12-22-99

A

Docket # PF-0049-2 DIV

"Express Mail" mailing label number EL 315 815 124 US. I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Assistant Commissioner for Patents, Box PATENT APPLICATION, Washington, D.C. 20231 on 12-10-99

By: Nancy Ramos

Printed: Nancy Ramos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.53(b)

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Dear Sir:

This is a request for filing a **DIVISIONAL** application under 37 CFR 1.53(b) of pending prior application Serial No. 09/196,480, filed on November 19, 1998, which is a divisional application of U.S. application Serial No. 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393, both entitled A NOVEL HUMAN JAK2 KINASE.

1. X Enclosed is a copy of the prior application, U.S. Application Serial No. 08/567,508 filed December 5, 1995, including the oath or declaration as originally signed.

2. X With regard to the requirement of 37 CFR 1.821(e) which requires that a copy of the Sequence Listing in computer readable form (CRF) be submitted, Applicants state that the computer readable form of the "Sequence Listing" for the instant divisional application is identical with the substitute sequence listing filed December 11, 1997, for Serial No. 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393, to which priority is claimed. In accordance with 37 C.F.R. §1.821(e), please use the computer readable form filed with the December 11, 1997 substitute sequence listing, as the computer readable form for the instant divisional application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant divisional application.

3. X Cancel in this application original claims 2, 3, and 11-13 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

4. X The inventors of the invention being claimed in this application are: Roger Coleman and Susan G. Stuart.

5. X In accordance with 37 CFR 1.63(d), a copy of the originally signed declaration showing applicants' signatures as filed on March 20, 1996 is enclosed.

6. X Amend the specification by inserting before the first line the sentence: "This application is a divisional application of U.S. application serial number 09/196,480, filed November 19, 1998, which is a divisional of U.S. application serial number 08/567,508, filed December 5, 1995, issued June 22, 1999, as U.S. Patent No. 5,914,393."

7. X The filing fee is calculated below:

Claims	Number Filed	Minus	Number Extra	Other Than Small Entity Rate	Fee	Basic Fee $760.00
Total Claims	13	-20	0	x $18		$0
Indep. Claims	1	-3	0	x $78		$0
__ Multiple Dependent Claim(s), if any				+ $260		$0

TOTAL FILING FEE $760.00

8. ___ An extension of time in the above-named prior application has been requested and the fees therefore have been authorized in said application.

9. X Please charge Incyte Pharmaceuticals, Inc. Deposit Account No. **09-0108** in the amount of $760.00 .

The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to Incyte Pharmaceuticals, Inc. Account No. **09-0108**.

A **duplicate** copy of this Request is enclosed.

10. X New formal drawings are enclosed.

11. X The prior application is assigned of record to Incyte Pharmaceuticals, Inc, recorded on March 25, 1996, at reel 7984/frame 0461.

12. X A preliminary amendment is enclosed.

13. X Also enclosed Return Postcard, Information Disclosure Statement, List of Cited References (1449), copy of previously submitted Certificate, Revocation of Power of Attorney and Appointment of New Attorneys, Submission of Formal Drawings, copy of Substitute Submission Under 37 C.F.R. 1.821-1.825, and copy of previously submitted Substitute Sequence Listing.

14. X The power of attorney of the prior application is to:

Narinder S. Banait	**Reg. No. 43,482**
Adam Warwick Bell	**Reg. No. 43,490**
Lucy J. Billings	**Reg. No. 36,749**
Michael C. Cerrone	**Reg. No. 39,132**
Diana Hamlet-Cox	**Reg. No. 33,302**
Colette C. Muenzen	**Reg. No. 39,784**
Lynn E. Murry	**Reg. No. 42,918**
Danielle M. Pasqualone	**Reg. No. 43,847**
Susan K. Sather	**Reg. No. 44,316**
David G. Streeter	**Reg. No. 43,168**

a. ___ An associate power of attorney is attached.

b. ___ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. X Address all future correspondence to:

INCYTE PHARMACEUTICALS, INC.
PATENT DEPARTMENT
3174 Porter Drive
Palo Alto, California 94304
Phone: (650) 855-0555, Fax: (650) 849-8886

Date: December 9, 1999 By: Susan K. Sather

Susan K. Sather
Reg. No. 44,316
Direct Dial Telephone: (650) 845-4646

___ Inventor(s)
___ Assignee of complete interest
X Attorney or agent of record
___ Filed under 37 CFR 1.34(a)
Registration number if acting under 37 CFR 1.34(a)_____.

"Express Mail" mailing label number EL 315 815 124 US. I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Assistant Commissioner for Patents, Box PATENT APPLICATION, Washington, D.C. 20231 on 12-10-99

By: Nancy Ramos

Printed: Nancy Ramos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING A PATENT APPLICATION UNDER 37 CFR 1.53(b)

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Indep. Claims	1	-3	0	x $78		$ 0
__ Multiple Dependent Claim(s), if any				+ $260		$ 0

TOTAL FILING FEE $ 760.00

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9. X Please charge Incyte Pharmaceuticals, Inc. Deposit Account No. **09-0108** in the amount of $760.00 .

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Lynn E. Murry	**Reg. No. 42,918**
Danielle M. Pasqualone	**Reg. No. 43,847**
Susan K. Sather	**Reg. No. 44,316**
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___ Inventor(s)
___ Assignee of complete interest
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___ Filed under 37 CFR 1.34(a)
Registration number if acting under 37 CFR 1.34(a) _____.

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By: Nancy Ramos

Printed: Nancy Ramos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: A NOVEL HUMAN JAK2 KINASE

Serial No.:	To Be Assigned	Filing Date:	Herewith
Examiner:	To Be Assigned	Group Art Unit:	To Be Assigned

Assistant Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified application as indicated below.

IN THE SPECIFICATION

Please amend the specification as follows:

At page 1, line 7, replace "prevention" with --prevention,--,

At page 1, line 15, replace "Jak3" with --Jak3,--.

At page 1, line 16, replace "addition" with --addition,--.

At page 3, line 23, replace "acid acid" with --acid--.

At page 3, line 24, replace "prevention" with --prevention,--.

At page 3, line 30, replace "(SEQ ID No 1)" with --(SEQ ID NO: 1)--.

At page 3, line 30 replace "(SEQ ID No 2)" with --(SEQ ID NO: 2)--

At page 3, line 30, replace "hjak2," with --hjak2--.

At page 3, line 30, delete "(SEQ ID No 2),".

At page 3, line 31, replace "HJAK2" with -- HJAK2 (SEQ ID NO:2)--.

At page 3, line 33, replace "86:1603-7)" with --86:1603-7),--.

At page 4, line 1, replace "hjak2, SEQ ID No1" with --hjak2 (SEQ ID NO:1)--.

At page 5, line 2, replace "Figure 1 displays" with --Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, and 1H display--.

At page 5, line 2, replace "nucleic acid and amino acid" with --nucleic acid (SEQ ID NO:1) and amino acid (SEQ ID NO:2)--.

At page 5, line 3, replace "this and the" with --these and in the--.

At page 5, line 4, replace "figure" with --figures--.

At page 5, line 5, replace "Inc" with --Inc.--.

At page 5, line 6, replace "Figure 2 shows" with --Figures 2A, 2B, 2C, 2D, and 2E show--.

At page 5, lines 6 through 7, replace "MUSPTK1 GI and HJAK2" with --HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3)--.

At page 5, line 12, replace "RNA" with --RNA,--.

At page 5, line 12, replace "sequence" with --sequence,--.

At page 8, lines 10 and 11, replace "a "fragment ", "portion ", or "segment" of" with --a "fragment", a "portion", or a "segment" of--.

At page 8, line 25, replace "etc" with --etc.--.

At page 8, line 26, replace "etc) or" with --etc.),--.

At page 8, line 26, replace "simians, etc)" with --simians, etc.)--.

At page 9, line 22, replace "an" with --a--.

At page 9, line 28, replace "86:1603-7)" with --86:1603-7),--.

At page 10, line 2, replace "including but not limited to" with --including, but not limited to,--.

At page 10, line 5, replace "produced and some" with --produced. Some--.

At page 10, line 20, delete "(**REF**)".

At page 10, line 28, replace "NY" with --NY, Chapters 4, 8, 16, and 17--.

At page 10, line 30, replace "City) ." with --New York NY, Chapters 9, 13, and 16).--.

At page 11, line 1, replace "PCR" with --PCR,--.

At page 11, line 6, replace "antisense" with --antisense orientation--.

At page 11, line 13, replace "Gobinda et al" with --Sarkar--.

At page 11, line 20, replace "Gobinda et al" with --Sarkar--.

At page 11, line 35, replace "Gobinda et al" with --Sarkar--.

At page 12, line 8, replace "PromoterFinder™" with --PROMOTERFINDER--.

At page 12, line 16, replace "XL-PCR™" with --XL-PCR--.

At page 12, line 26, replace "In step 2," with --In the second step,--.

At page 12, lines 27 and 28, replace "Steps 3 and 4," with --The third and fourth steps,--.

At page 12, line 29, replace "Step 5," with --The fifth step,--.

At page 12, line 30, replace "In step 6," with --The sixth step,--.

At page 13, line 16, replace "devise" with --device--.

At page 13, line 18, replace "Genotyper™" with --GENOTYPER--.

At page 13, line 18, replace "Sequence Navigator™" with --SEQUENCE NAVIGATOR--.

At page 13, line 36, replace "NO 1" with --NO:1--.

At page 14, line 9, replace "T7, T3 or SP6 and labelled" with --T7, T3, or SP6, and labeled--.

At page 16, line 29, after "using" insert --an--.

At page 16, line 30, replace "Peptide Synthesizer" with --peptide synthesizer--.

At page 17, line 32, replace "Library" with --The cDNA library--.

At page 17, lines 32 and 33, delete "obtained from Mayo Clinic".

At page 18, line 5, replace "Uni ZAP™" with --UNIZAP--.

At page 18, line 7, replace "pBluescript™" with --PBLUESCRIPT--.

At page 18, line 7, replace "library" with --library,--.

At page 18, line 8, replace ", and" with --and--.

At page 18, line 10, replace "XL1-Blue™" with --XL1-BLUE--.

At page 18, line 10, replace "pBluescript" with --PBLUESCRIPT--.

At page 18, line 20, replace "Plasmid Purification System" with --plasmid purification system--.

At page 18, line 25, replace "Catalyst" with --CATALYST--.

At page 18, line 26, replace "Hamilton Micro Lab" with --MICROLAB--.

At page 18, line 27, replace "Thermal Cyclers" with --thermal cyclers--.

At page 18, line 28, replace "Sequencing Systems" with --sequencing systems--.

At page 18, line 35, replace "Sequenase®" with --SEQUENASE--.

At page 18, line 35, replace "Corp)" with --Corp.,--.

At page 19, line 5, delete "Hamilton".

At page 19, line 6, replace "Micro Lab" with --MICROLAB--.

At page 19, line 6, replace "Thermal Cycler" with --thermal cycler--.

At page 19, line 7, replace "Catalyst" with --CATALYST--.

At page 19, line 19, replace "INHERIT™ 670 Sequence Analysis System" with --INHERIT 670 sequence analysis system--.

At page 19, line 31, replace "INHERIT™" with --INHERIT--.

At page 20, line 24, replace "Fig 1" with --Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, and 1H--.

At page 20, line 24, replace "Fig 2" with --Figures 2A, 2B, 2C, 2D, and 2E--.

At page 20, line 37, replace "Oligo" with --OLIGO--.

At page 21, lines 5 and 6, replace "XLR = GGGCGGAAGTGCTCTCGGCGGAAG" with --XLR (SEQ ID NO:4)--.

At page 21, line 6, replace "XLF = AGTGTGCTACAGTGCTGGTCGTCG" with --XLF (SEQ ID NO:5)--.

At page 21, line 13, replace "Thermal Cycler" with --thermal cycler--.

At page 21, line 35, replace " QIAQuick™" with --QIAQUICK--.

At page 22, line 34, replace "229-36" with --212:229-236--.

At page 23, line 17, replace "Oligo 4.0" with --OLIGO 4.0 software--.

At page 23, line 20, replace "Inherit Analysis" with --INHERIT analysis--.

At page 24, line 4, replace "a organ, tumor, synovial cavity" with --an organ, a tumor, a synovial cavity,--.

At page 24, line 8, replace "and three" with --and for three--.

At page 24, line 12, replace "tissue" with --tissue,--.

At page 24, line 19, replace "tissue" with --tissue,--.

At page 25, line 9, replace "then used" with --then be used--.

At page 25, line 33, replace "tac" with --tac)--.

At page 25, line 34, replace "oxidase" with --oxidase,--.

At page 27, line 35, replace "regions, as shown in Fig. 3," with --regions--.

At page 28, line 1, replace "N-terminus" with --N-terminus,--.

At page 28, line 2, delete "those".

At page 28, line 5, replace "peptides," with --peptides of--.

At page 28, line 5, replace "length," with --length--.

At page 28, line 6, replace "Peptide Synthesizer Model 431A" with "431A peptide synthesizer--.

At page 30, line 8, replace "Sepharose" with --SEPHAROSE--.

Please replace pages 35 through 43 with substitute pages 35 through 43, which accompany this Preliminary Amendment.

IN THE DRAWINGS

Please amend Figures 2A, 2B, 2C, 2D, 2E, and 2F as shown in red on the attached sheets.

Please replace Figures 1A, 1B, 1C, 1D, 1E, and 1F and Figures 2A, 2B, 2C, 2D, 2E, 2F as filed with the formal drawings which accompany this Preliminary Amendment.

IN THE CLAIMS

Please cancel claims 2, 3 and 11-13.

REMARKS

Justification for the amendments is as follows. Claims 2, 3, and 11-13 are canceled above, and claims 1, 4-10, and 14-18 are pending.

The amendment to the specification at pages 35 through 43 of the specification replaces the Sequence Listing as filed with the Substitute Sequence Listing filed December 11, 1997, in prior U.S. application Serial No. 08/567,508, and referenced in the filing papers for the present application. (See page 1 of the transmittal for the present application.) The Substitute Sequence Listing properly identifies the MUSPTK1 sequence, described at page 5 of the specification and shown in Figures 2A, 2B, 2C, 2D, 2E, and 2F as originally filed, as SEQ ID NO:3, and identifies sequences XLR and XLF, which appear at page 21 of the specification as originally filed, as SEQ ID NO:4 and SEQ ID NO:5, respectively. These sequences do not constitute new matter as MUSPTK1, XLR, and XLF appear in the application as filed.

The Substitute Sequence Listing corrects an error in SEQ ID NO:2 in the Sequence Listing as originally filed. Specifically, the latter part of SEQ ID NO:2, starting around residue 990, appears to have been incorrectly translated. However, SEQ ID NO:2 was correct as shown in Figures 1A, 1B, 1C, 1D, 1E, and 1F as filed. Accordingly, SEQ ID NO:2 as it appears in the Substitute Sequence Listing accompanying this Amendment is correct. This corrected sequence is not new matter as the correct sequence was shown in Figures 1A, 1B, 1C, 1D, 1E, and 1F of the application as filed. Applicants apologize for any inconvenience that might have been caused by this error. Applicants note that SEQ ID NO:1 was correct in the application as filed in both Figures 1A, 1B, 1C, 1D, 1E, and 1F and in the Sequence Listing.

The amendments to the specification at page 5, lines 2 and 6, and page 20, line 24, are made to reflect the renumbering of the figures in the preparation of formal drawings. The remaining amendments to the specification are merely typographical or grammatical in nature.

The amendments to the drawings were made to clarify Figures 2A, 2B, 2C, 2D, and 2E, and 2F. Specifically, Figures 2A, 2B, 2C, 2D, 2E, and 2F as originally filed showed the alignment between HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3), along with two consensus sequences generated by DNASTAR software, the multisequence alignment program used. Figures 2A, 2B, 2C, 2D, 2E, and 2F as amended show only the alignment

between HJAK2 (SEQ ID NO:2) and MUSPTK1 (GI 409584; SEQ ID NO:3).

No new matter is added by any of these amendments.

If there are any questions regarding the above, the Examiner is invited to call Applicants' Agent at (650) 855-0555.

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Date: December 9, 1999

Susan K. Sather

Susan K. Sather
Reg. No. 44,316
Direct Dial Telephone: (650) 845-4646

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Phone: (650) 855-0555
Fax: (650) 849-8886

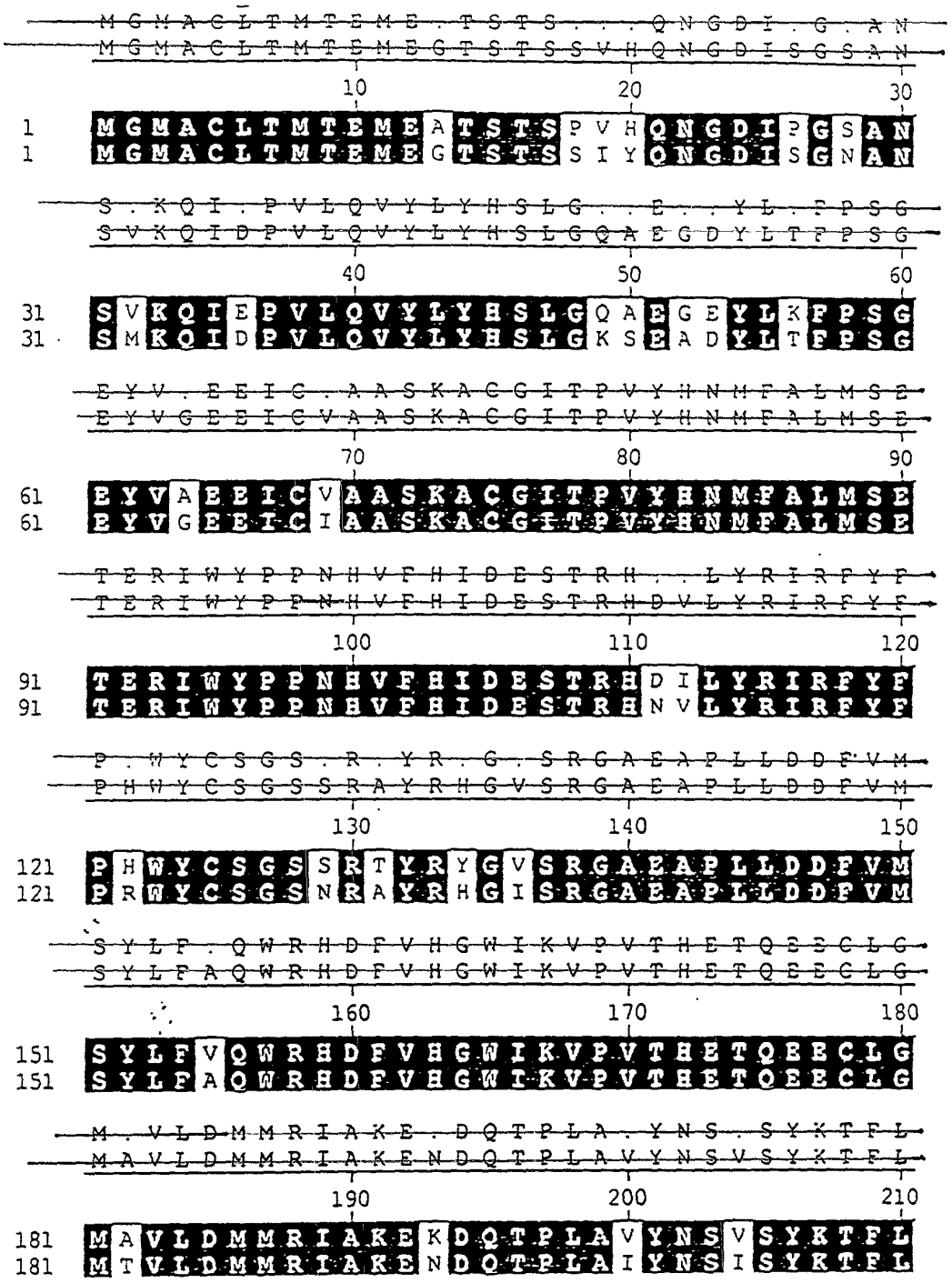

FIGURE 2A

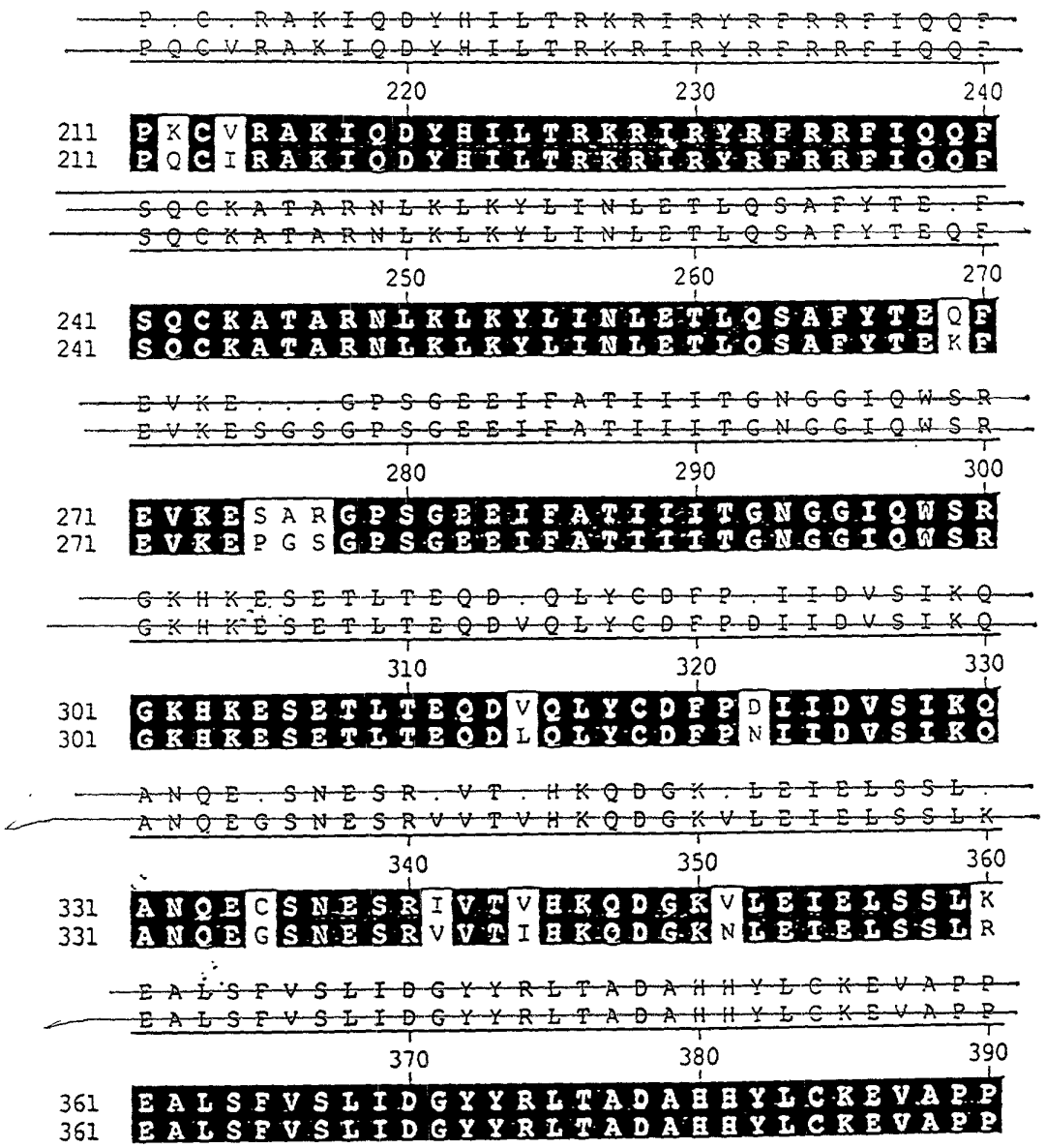
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FIGURE 2B

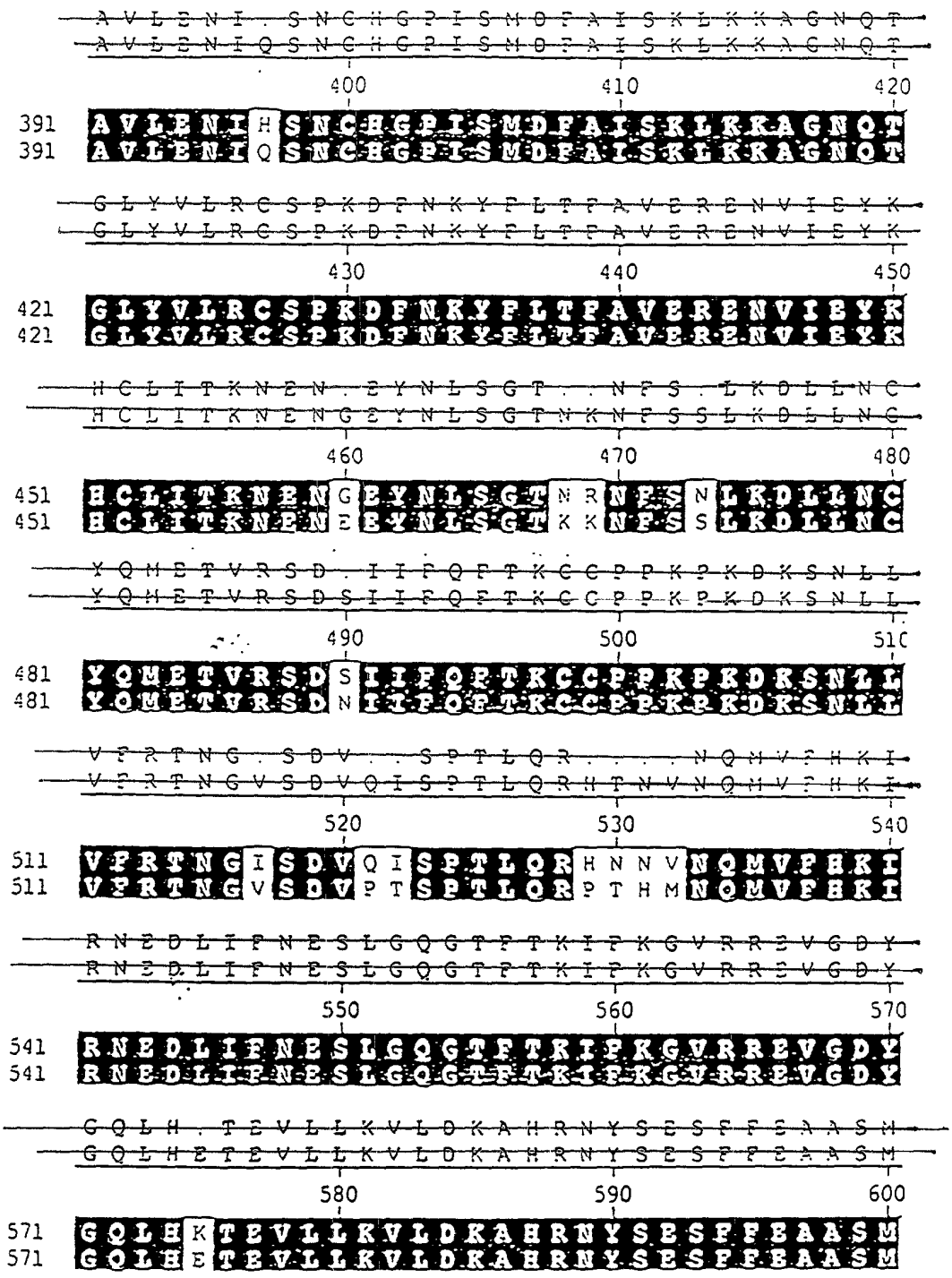
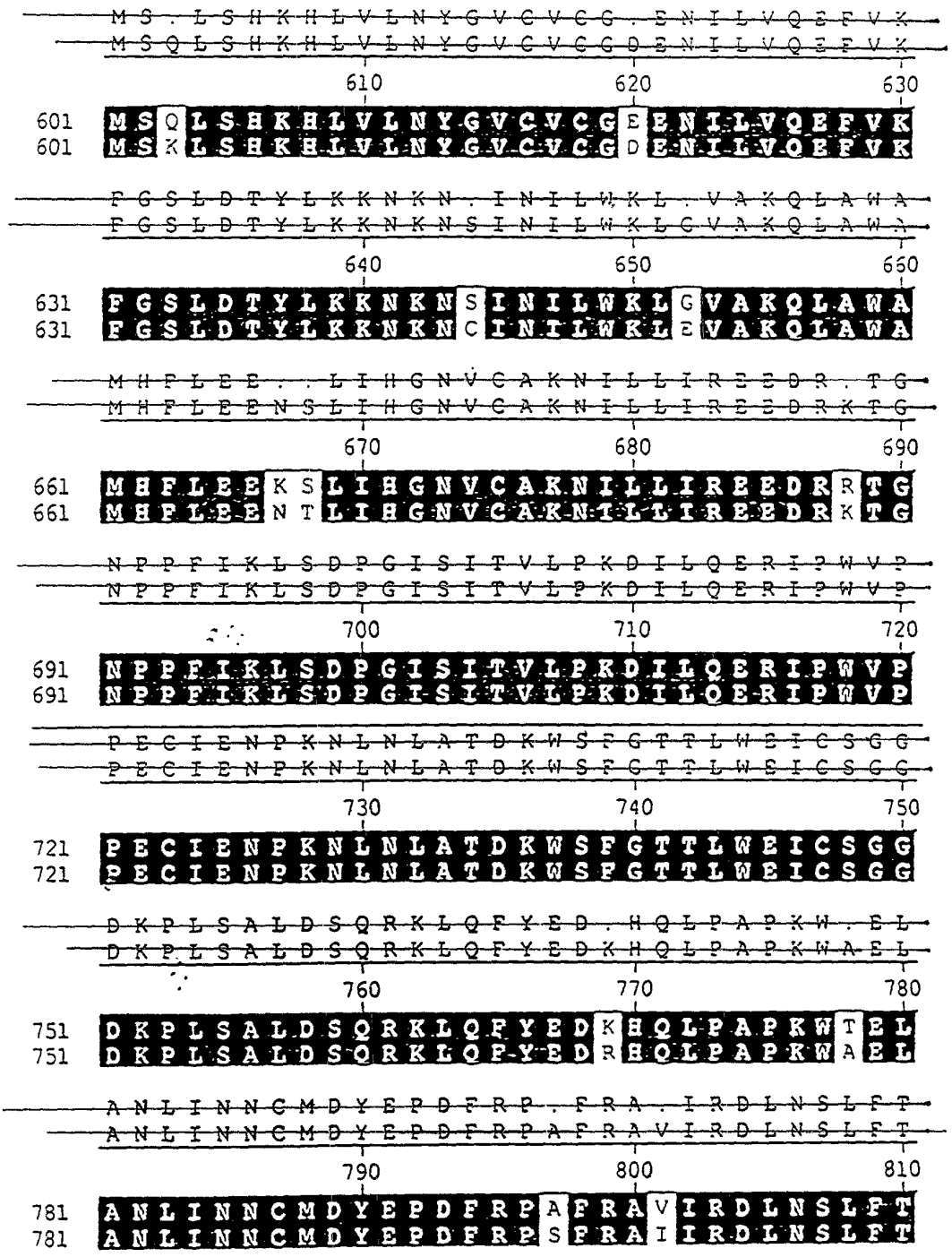


FIGURE 2C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780
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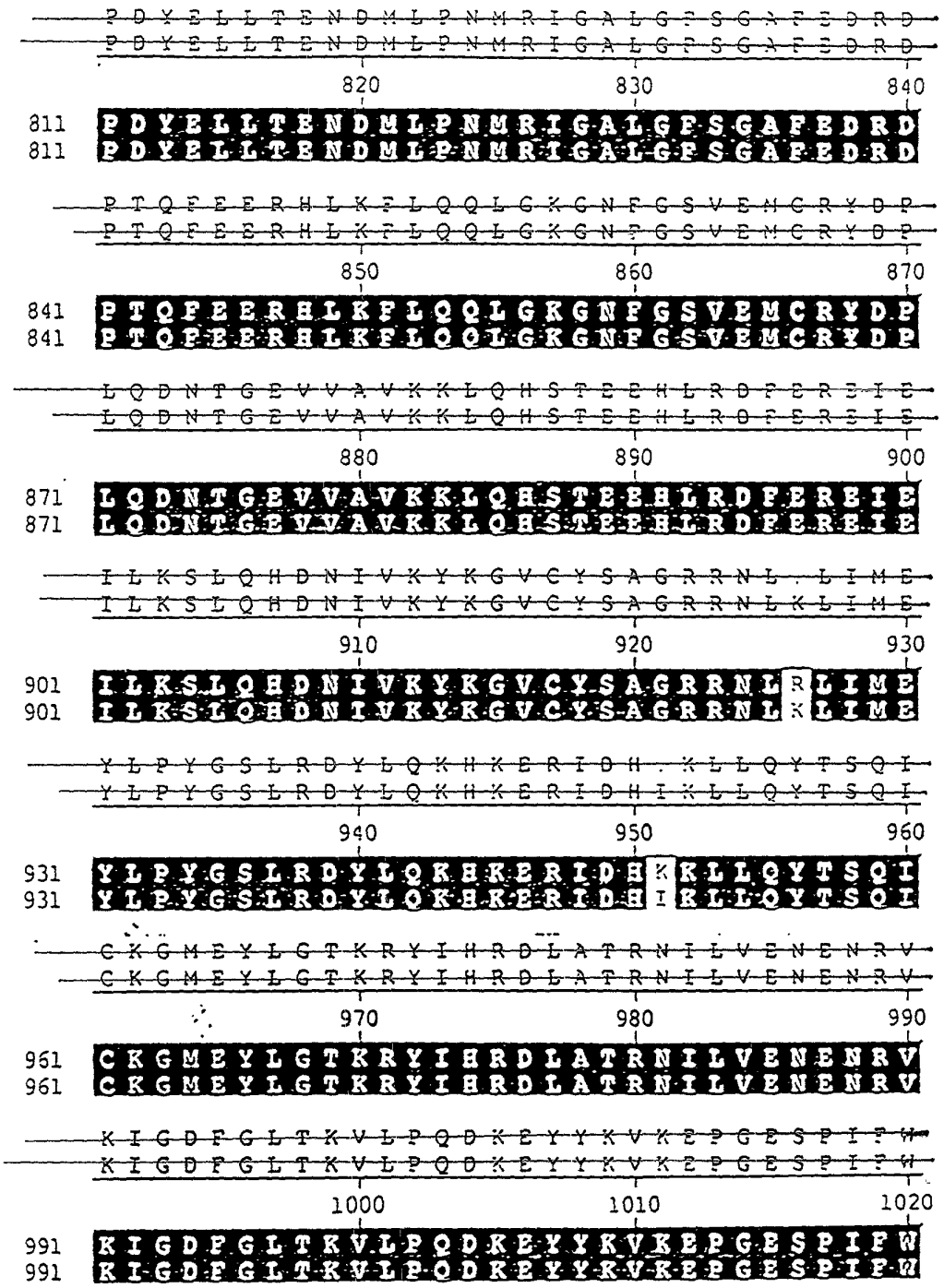
FIGURE 2D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980
990
961 CKGMEYLGTKRYIHRDLATRNILVENENRV
961 CKGMEYLGTKRYIHRDLATRNILVENENRV
1000
1010
1020
991 KIGDFGLTKVLPQDKEYYKVKEPGESPIFW
991 KIGDFGLTKVLPQDKEYYKVKEPGESPIFW

FIGURE 2E

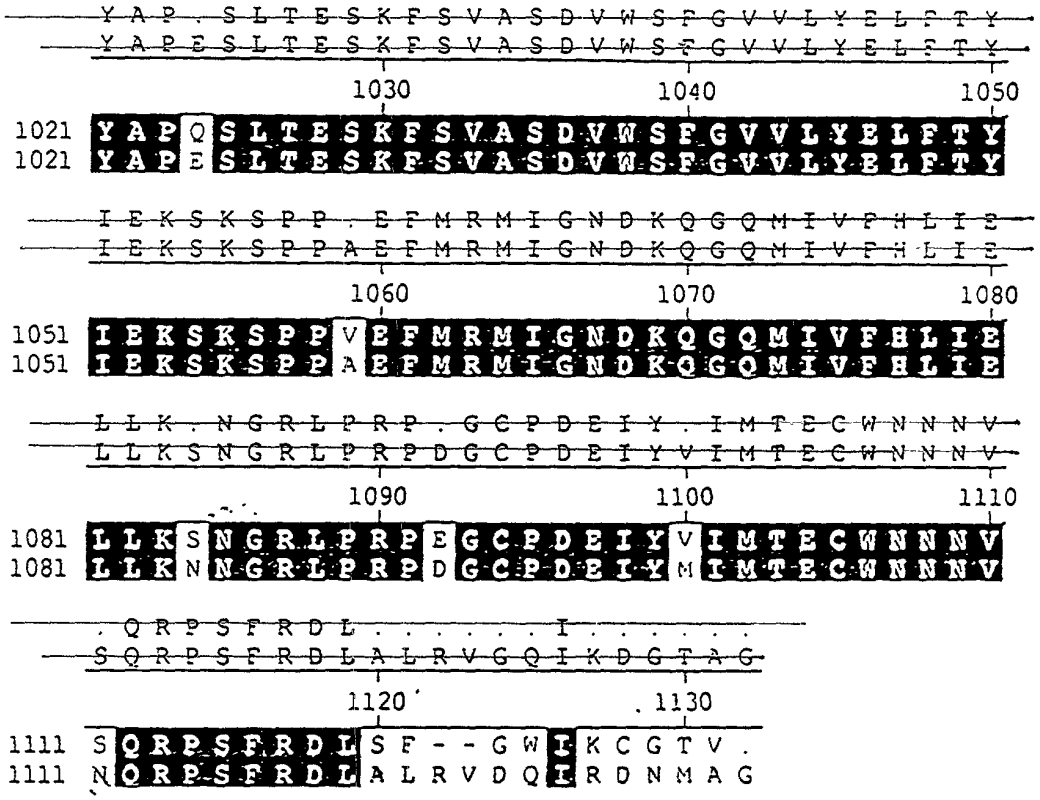
1030
1040
1050
1021 YAPQSLTESKFSVASDVWSFGVVLYELFTY
1021 YAPESLTESKFSVASDVWSFGVVLYELFTY
1060
1070
1080
1051 IEKSKSPPVEFMRMIGNDKQGQMIVFHLIE
1051 IEKSKSPPAEFMRMIGNDKQGQMIVFHLIE
1090
1100
1110
1081 LLKSNGRLPRPEGCPDEIYVIMTECWNNNV
1081 LLKNNGRLPRPDGCPDEIYMIMTECWNNNV
1120
1130
1111 SQRPSFRDLSF--GWIKCGTV.
1111 NQRPSFRDLALRVDQIRDNMAG

FIGURE 2F

PF-0049P

A NOVEL HUMAN JAK2 KINASE

FIELD OF THE INVENTION

The present invention relates to a novel, human Jak2 kinase isolated from human placenta and to the use of this novel protein and its nucleic acid sequence in the diagnosis, study, prevention and treatment of disease.

BACKGROUND OF THE INVENTION

JAK kinases are Janus family nonreceptor protein-tyrosine kinases (NR-PTK) that lack transmembrane regions and form functional complexes with the intercellular regions of other cell surface receptors. They were first identified as the products of mutant oncogenes in cancer cells where their activation was no longer subject to normal cellular controls. Described JAK kinases include Jak1, Jak2, and Jak3 which all share the conserved kinase domain. In addition these proteins have 5 to 100 amino acid residues located on either side of, or inserted into loops of, the carboxyterminal kinase domain which allow the regulation of each kinase as it recognizes and interacts with its target protein. Known target proteins include growth hormone receptor, prolactin receptor, erythropoietin receptor, cytokine receptors and others which utilize the common chain known as gp130. These receptors are unique both in their ability to recruit multiple PTKs and in the diversity of their responses within different cell types (Taniguchi T (1995) Science 268:251-55). Genetic evidence places these kinases in the interferon α and γ signal transduction pathways which are widely expressed in mammalian cells.

Kinases regulate many different cell proliferation, differentiation, and signaling processes by adding phosphate groups to proteins. The high energy phosphate which drives activation is generally transferred from adenosine triphosphate molecules (ATP) to a particular protein by the PTKs, and the transfer process is roughly analogous to turning on a molecular switch. When the switch goes on, the kinase activates a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, transcription factor, or another kinase. For example, in their normal role, the JAK NR-PTKs are capable of regulating tyrosine phosphorylation of STAT proteins, signal transducers and activators of transcription, such that they translocate to the nucleus and bind DNA (David M et al. (1995)

Science 269:1721-1723). In contrast, uncontrolled kinase signaling has been implicated in inflammation, oncogenesis, arteriosclerosis, and psoriasis.

Almost all kinases contain a similar 250-300 amino acid catalytic domain. The N-terminal domain generally folds into a two-lobed structure to bind and orient ATP (or GTP) donor molecules. The larger C terminal lobe binds the protein substrate and carries out the transfer of phosphate from ATP to the hydroxyl group of a tyrosine residue. The primary structure of the kinase domain is conserved in the residues: G_{50} and G_{52} in subdomain I, K_{72} in subdomain II, G_{91} in subdomain III, E_{208} in subdomain VIII, D_{220} and G_{225} in subdomain IX, and the amino acid motifs of subdomains VIB, VIII and IX (Hardie G and Hanks S (1995) Academic Press, San Diego CA).

The novel human Jak2 kinase, hjak2, of the present application shows significant conservation of the diagnostic kinase residues which allowed its identification from among the isolated cDNAs of a placenta library, the anatomy and physiology of which is briefly described below.

The placenta is a thickened disk-shaped temporary organ that interchanges gases, nutrients, hormones, excretory products, humoral antibodies (IgG), and any other circulating substances between the maternal and fetal bloodstreams. Receptors facilitate the transport of glucose, amino acids, and IgG directly from maternal blood to fetal blood. The placenta is the only organ composed of cells derived from two individuals, the fetal extraembryonic chorion and the maternal endometrium. The boundary between these two tissues is marked by extracellular products of necrosis referred to as fibrinoid. This boundary results from the various tissue interactions, immunological responses, etc. which occur in the placenta.

The major tissue interaction involves the expression of paternal antigens by the chorionic villi which is directly adjacent to maternal blood. Although the mother initiates an immunological response, fetal tissue is not typically rejected. This is attributed to the fact that the fetus only expresses major histocompatibility complex (MHC) I, and not MHC II which is the major cause of organ allograft rejection. In addition, uterine secretions during early gestation contain significant amounts of glucose and glycoproteins which may participate in local immunosuppression.

Although infections by bacteria, viruses, mycoplasmas, or parasites may ascend from the endocervical canal or reach the placenta through maternal blood, they rarely cause gross pathological changes because of maternal immune defense.

Soon after implantation, fetal villi begin to control maternal physiology to create an optimal environment for development. This involves the production of chorionic gonadotropin, estrogen and progesterone, chorionic somatomammotropin, insulin-like growth factors, platelet derived growth factor, prolactin, and various cytokines. These and other factors such as hjak2 certainly regulate the numerous activities (respiratory, immunological, gastrointestinal, and urinary) which occur within the placenta and between maternal and fetal tissues.

The anatomy and physiology of human placenta is reviewed, inter alia, in Benirschke and Kaufmann, (1992) Pathology of the Human Placenta, Springer-Verlag, New York NY, pp. 542-635; Herrera Gonzalez and Dresser (1993) Dev Comp Immunol 17(1):1-18; Mitchell et al. (1993) Placenta 14:249-275; Naeye (1992) Disorders of the Placenta, Fetus, and Neonate: Diagnosis and Clinical Significance, Moseby Year Book, St. Louis MO; and Rutanen (1993) Ann Med 25:343-347.

SUMMARY

The present invention relates to a novel human Jak2 kinase and to the use of the protein and its nucleic acid acid sequence in the study, diagnosis, prevention and treatment of diseases. Human Jak2 kinase (hjak2) was first identified as a partial nucleotide sequence in Incyte Clone 179527 during a computer search for nucleotide sequence alignments among the cDNAs of a placenta library. A modified XL-PCR procedure, specially designed oligonucleotides, and cDNAs of the placenta library were used to extend Incyte Clone 179527 to full length. The assembled nucleotide sequence (SEQ ID No 1), hjak2, encodes the polypeptide (SEQ ID No 2), HJAK2. Computer search and alignment of the full length amino acid sequence showed that HJAK2 has 92% similarity to murine Jak2 kinase (MUSPTK1; GenBank GI 409584; Wilks AF (1989) Proc Nat Acad Sci 86:1603-7) which in turn has 96% sequence similarity with human Jak1 kinase. These homologies and the conserved residues, G_{48}, K_{73}, E_{192}, and D_{220} which all lie within the catalytic domain contributed to the naming and uses of hjak2.

The complete nucleic acid sequence encoding hjak2, SEQ ID No1 disclosed herein, provides the basis for the design of antisense molecules useful in diminishing or eliminating expression of the genomic nucleotide sequence. For example, hjak2, or its oligonucleotides, fragments, portions, or complement, may be used in diagnostic hybridization or amplification assays of biopsied tissue to detect and/or quantify abnormalities in gene expression associated with an immunological disorder. The present invention also relates, in part, to the inclusion of the nucleic acid sequence in an expression vector which can be used to transform host cells or organisms. Such transgenic hosts are useful for production and recovery of the encoded HJAK2.

The invention further comprises using purified HJAK2 polypeptide to produce antibodies or to identify antagonists or inhibitors which bind HJAK2. Anti-HJAK antibodies may be used in membrane, tissue-based or ELISA technologies to detect any disease state or condition related to the aberrant expression of HJAK2. Antibodies, antagonists or inhibitors can be used to bind HJAK2 preventing the transfer of high energy phosphate molecules and therefore signal transduction. The invention also comprises pharmaceutical compositions containing the peptide, antibodies, antagonists or inhibitors for the diagnosis, prevention or treatment of conditions associated with altered or uncontrolled hjak2 expression. These conditions may include, but are not limited to: arteriosclerosis, asthma, bronchitis, emphysema, inflammatory bowel disease, leukemia, oncogenesis, osteoarthritis, psoriasis, rheumatoid arthritis, septic shock, and systemic lupus erythematosus. Steps for testing a biological sample with probes, oligomers, fragments or portions of the hjak2 nucleotide sequence or antibodies produced against the purified HJAK2 protein are provided.

Antisense molecules, antibodies, antagonists or inhibitors (including proteins, peptides, oligopeptides or organic molecules capable of compromising or modulating HJAK2 expression) may also be used for therapeutic purposes, for example, in neutralizing the abberrent activity of a HJAK2 associated with, for example, inflammation or oncogenesis. The present invention also provides for pharmaceutical compositions for the treatment of disease states associated with aberrant expression of hjak2 comprising the forementioned antisense molecules, antibodies, antagonists or inhibitors.

DESCRIPTION OF THE FIGURES

Figure 1 displays an alignment of the nucleic acid and amino acid sequences of human jak2 kinase. Alignments shown in this and the following figure were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 2 shows the amino acid sequence similarity between MUSPTK1, GI and HJAK2.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the abbreviation for the novel human Jak2 kinase in lower case (hjak2) refers to a gene, cDNA, RNA or nucleic acid sequence while the upper case version (HJAK2) refers to a protein, polypeptide, peptide, oligopeptide, or amino acid sequence.

An "oligonucleotide" or "oligomer" is a stretch of nucleotide residues which has a sufficient number of bases to be used in a polymerase chain reaction (PCR). These short sequences are based on (or designed from) genomic or cDNA sequences and are used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

"Probes" are nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligonucleotides. They may be single- or double-stranded and are carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

"Reporter" molecules are chemical moieties used for labelling a nucleic or amino acid sequence. They include, but are not limited to, radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents. Reporter molecules associate with, establish the presence of, and may allow quantification of a particular nucleic or amino acid sequence.

A "portion" or "fragment" of a polynucleotide or nucleic acid comprises all or any part of the nucleotide sequence having fewer nucleotides than about 6 kb, preferably fewer than about 1 kb which can be used as a probe. Such probes may be labelled with reporter molecules using nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. After pretesting to optimize reaction conditions and to eliminate false positives, nucleic acid probes may be used in Southern, northern or in situ hybridizations to determine whether DNA or RNA encoding the protein is present in a biological sample, cell type, tissue, organ or organism.

"Recombinant nucleotide variants" are polynucleotides which encode a protein. They may be synthesized by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce specific restriction sites or codon usage-specific mutations, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic host system, respectively.

"Linkers" are synthesized palindromic nucleotide sequences which create internal restriction endonuclease sites for ease of cloning the genetic material of choice into various vectors. "Polylinkers" are engineered to include multiple restriction enzyme sites and provide for the use of both those enzymes which leave 5' and 3' overhangs such as BamHI, EcoRI, PstI, KpnI and Hind III or which provide a blunt end such as EcoRV, SnaBI and StuI.

"Control elements" or "regulatory sequences" are those nontranslated regions of the gene or DNA such as enhancers, promoters, introns and 3' untranslated regions which interact with cellular proteins to carry out replication, transcription, and translation. They may occur as boundary sequences or even split the gene. They function at the molecular level and along with regulatory genes are very important in development, growth, differentiation and aging processes.

"Chimeric" molecules are polynucleotides or polypeptides which are created by combining one or more of nucleotide sequences of this invention (or their parts) with additional nucleic acid sequence(s). Such combined sequences may be introduced into an appropriate vector and expressed to

give rise to a chimeric polypeptide which may be expected to be different from the native molecule in one or more of the following characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signalling, etc.

"Active" refers to those forms, fragments, or domains of an amino acid sequence which display the biologic and/or immunogenic activity characteristic of the naturally occurring peptide.

"Naturally occurring HJAK2" refers to a polypeptide produced by cells which have not been genetically engineered or which have been genetically engineered to produce the same sequence as that naturally produced. Specifically contemplated are various polypeptides which arise from post-transnational modifications. Such modifications of the polypeptide include but are not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

"Derivative" refers to those polypeptides which have been chemically modified by such techniques as ubiquitination, labelling (see above), pegylation (derivatization with polyethylene glycol), and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins.

"Recombinant polypeptide variant" refers to any polypeptide which differs from naturally occurring HJAK2 by amino acid insertions, deletions and/or substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing characteristics of interest may be found by comparing the sequence of HJAK2 with that of related polypeptides and minimizing the number of amino acid sequence changes made in highly conserved regions.

Amino acid "substitutions" are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid "insertions" or "deletions" are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. The variation allowed in a particular amino acid sequence may be experimentally determined by producing the peptide synthetically or by systematically making insertions, deletions, or substitutions of

nucleotides in the hjak2 sequence using recombinant DNA techniques.

A "signal or leader sequence" is a short amino acid sequence which or can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

An "oligopeptide" is a short stretch of amino acid residues and may be expressed from an oligonucleotide. It may be functionally equivalent to and either the same length as or considerably shorter than a "fragment ", "portion ", or "segment" of a polypeptide. Such sequences comprise a stretch of amino acid residues of at least about 5 amino acids and often about 17 or more amino acids, typically at least about 9 to 13 amino acids, and of sufficient length to display biologic and/or immunogenic activity.

An "inhibitor" is a substance which retards or prevents a chemical or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives.

A "standard" is a quantitative or qualitative measurement use for comparison. Preferably, it is based on a statistically appropriate number of samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles. The samples of a particular standard may be normal or similarly abnormal.

"Animal" as used herein may be defined to include human, domestic (cats, dogs, etc), agricultural (cows, horses, sheep, goats, chicken, fish, etc) or test species (frogs, mice, rats, rabbits, simians, etc).

"Conditions" includes cancers, disorders or diseases in which hjak2 activity may be implicated. These specifically include, but are not limited to, anemia, arteriosclerosis, asthma, bronchitis, emphysema, gingivitis, inflammatory bowel disease, insulin-dependent diabetes mellitus leukemia, multiple endocrine neoplasias, osteoarthritis, osteoporosis, pulmonary fibrosis, rheumatoid arthritis, septic shock syndromes, and systemic lupus erythematosus.

Since the list of technical and scientific terms cannot be all encompassing, any undefined terms shall be construed to have the same meaning as is commonly understood by one of skill in the art to which this

invention belongs. Furthermore, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to a "restriction enzyme" or a "high fidelity enzyme" may include mixtures of such enzymes and any other enzymes fitting the stated criteria, or reference to the method includes reference to one or more methods for obtaining cDNA sequences which will be known to those skilled in the art or will become known to them upon reading this specification.

Before the present sequences, variants, formulations and methods for making and using the invention are described, it is to be understood that the invention is not to be limited only to the particular sequences, variants, formulations or methods described. The sequences, variants, formulations and methodologies may vary, and the terminology used herein is for the purpose of describing particular embodiments. The terminology and definitions are not intended to be limiting since the scope of protection will ultimately depend upon the claims.

Description of the Invention

The present invention provides for a purified polynucleotide which encodes a novel human Jak2 kinase which is expressed in human cells or tissues. The human Jak2 kinase (hjak2; Incyte Clone 179527) was first identified among the cDNAs from an placenta cDNA library. The naming and proscribed uses of the present invention are based in part on the conserved residues found in HJAK2. These particularly include the residues G_{48}, K_{73}, E_{192}, and D_{220}, which are all found within the catalytic domain. Computer search and alignment of the full length amino acid sequences showed that HJAK2 has 92% similarity to murine Jak2 kinase (MUSPTK1; GenBank GI 409584; Wilks AF (1989) Proc Nat Acad Sci 86:1603-7) which in turn has 96% sequence similarity with human Jak1 kinase.

Purified nucleotide sequences, such as hjak2, have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include their use as PCR or hybridization probes, for chromosome and gene mapping, in the production of sense or antisense nucleic acids, in screening for new therapeutic molecules, etc. These examples are well known and are not intended to be limiting. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the

new techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair interactions.

As a result of the degeneracy of the genetic code, a multitude of HJAK2-encoding nucleotide sequences may be produced and some of these will bear only minimal homology to the endogenous sequence of any known and naturally occurring Jak2 kinase sequence. This invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HJAK2 and all such variations are to be considered as being specifically disclosed.

Although the hjak2 nucleotide sequence and its derivatives or variants are preferably capable of identifying the nucleotide sequence of the naturally occurring HJAK2 under optimized conditions, it may be advantageous to produce HJAK2-encoding nucleotide sequences possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host (**REF**). Other reasons for substantially altering the nucleotide sequence encoding the HJAK2 without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a longer half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding HJAK2 may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques (Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York City). Useful sequences for joining to hjak2 include an assortment of cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and the like. Vectors of interest include vectors for replication, expression, probe generation, sequencing, and the like. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for one or more host cell systems.

PCR as described in US Patent Nos. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the hjak2 nucleotide sequence. Such oligomers are generally chemically synthesized, but they may be of recombinant origin or a mixture of both. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'->3') and one with antisense (3' to 5') employed under optimized conditions for identification of a specific gene or diagnostic use. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for identification and/or quantitation of closely related DNA or RNA sequences.

Full length genes may be cloned utilizing partial nucleotide sequence and various methods known in the art. Gobinda et al (1993; PCR Methods Applic 2:318-22) disclose "restriction-site PCR" as a direct method which uses universal primers to retrieve unknown sequence adjacent to a known locus. First, genomic DNA is amplified in the presence of primer to linker and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase. Gobinda et al present data concerning Factor IX for which they identified a conserved stretch of 20 nucleotides in the 3' noncoding region of the gene.

Inverse PCR is the first method to report successful acquisition of unknown sequences starting with primers based on a known region (Triglia T et al(1988) Nucleic Acids Res 16:8186). The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template. Divergent primers are designed from the known region. The multiple rounds of restriction enzyme digestions and ligations that are necessary prior to PCR make the procedure slow and expensive (Gobinda et al, supra).

Capture PCR (Lagerstrom M et al (1991) PCR Methods Applic 1:111-19) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and YAC DNA. As noted by Gobinda et al (supra), capture PCR also requires multiple restriction enzyme digestions and ligations to

place an engineered double-stranded sequence into an unknown portion of the DNA molecule before PCR. Although the restriction and ligation reactions are carried out simultaneously, the requirements for extension, immobilization and two rounds of PCR and purification prior to sequencing render the method cumbersome and time consuming.

Parker JD et al (1991; Nucleic Acids Res 19:3055-60), teach walking PCR, a method for targeted gene walking which permits retrieval of unknown sequence. In this same vein, PromoterFinder™ a new kit available from Clontech (Palo Alto CA) uses PCR and primers derived from p53 to walk in genomic DNA. Nested primers and special PromoterFinder libraries are used to detect upstream sequences such as promoters and regulatory elements. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

Another new PCR method, "Improved Method for Obtaining Full Length cDNA Sequences" by Guegler et al, Patent Application Serial No 08/487,112, filed June 7, 1995 and hereby incorporated by reference, employs XL-PCR™ (Perkin-Elmer, Foster City CA) to amplify and extend partial nucleotide sequence into longer pieces of DNA. This method was developed to allow a single researcher to process multiple genes (up to 20 or more) at one time and to obtain an extended (possibly full-length) sequence within 6-10 days. This new method replaces methods which use labelled probes to screen plasmid libraries and allow one researcher to process only about 3-5 genes in 14-40 days.

In the first step, which can be performed in about two days, any two of a plurality of primers are designed and synthesized based on a known partial sequence. In step 2, which takes about six to eight hours, the sequence is extended by PCR amplification of a selected library. Steps 3 and 4, which take about one day, are purification of the amplified cDNA and its ligation into an appropriate vector. Step 5, which takes about one day, involves transforming and growing up host bacteria. In step 6, which takes approximately five hours, PCR is used to screen bacterial clones for extended sequence. The final steps, which take about one day, involve the preparation and sequencing of selected clones.

If the full length cDNA has not been obtained, the entire procedure is repeated using either the original library or some other preferred library. The preferred library may be one that has been size-selected to

include only larger cDNAs or may consist of single or combined commercially available libraries, eg. lung, liver, heart and brain from Gibco/BRL (Gaithersburg MD). The cDNA library may have been prepared with oligo (dT) or random priming. Random primed libraries are preferred in that they will contain more sequences which contain 5' ends of genes. A randomly primed library may be particularly useful if an oligo (dT) library does not yield a complete gene. It must be noted that the larger and more complex the protein, the less likely it is that the complete gene will be found in a single plasmid.

A new method for analyzing either the size or the nucleotide sequence of PCR products is capillary electrophoresis. Systems for rapid sequencing are available from Perkin Elmer (Foster City CA), Beckman Instruments (Fullerton CA), and other companies. Capillary sequencing employs flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity is converted to electrical signal using appropriate software (eg. Genotyper™ and Sequence Navigator™ from Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display is computer controlled. Capillary electrophoresis provides greater resolution and is many times faster than standard gel based procedures. It is particularly suited to the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample. The reproducible sequencing of up to 350 bp of M13 phage DNA in 30 min has been reported (Ruiz-Martinez MC et al (1993) Anal Chem 65:2851-8).

Another aspect of the subject invention is to provide for hjak2 hybridization probes which are capable of hybridizing with naturally occurring nucleotide sequences encoding HJAK2. The stringency of the hybridization conditions will determine whether the probe identifies only the native nucleotide sequence of hjak2 or sequences of other closely related Jak2 kinase molecules. If degenerate hjak2 nucleotide sequences of the subject invention are used for the detection of related kinase encoding sequences, they should preferably contain at least 50% of the nucleotides of the sequences presented herein. Hybridization probes of the subject invention may be derived from the nucleotide sequence presented in SEQ ID NO 1 or from surrounding genomic sequences comprising untranslated regions

such as promoters, enhancers and introns. Such hybridization probes may be labelled with appropriate reporter molecules.

Means for producing specific hybridization probes for this Jak2 kinase include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled nucleotide. Alternatively, the cDNA sequence may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. A number of companies (such as Pharmacia Biotech, Piscataway NJ; Promega, Madison WI; US Biochemical Corp, Cleveland, OH; etc.) supply commercial kits and protocols for these procedures.

It is also possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. Sometimes the source of information for producing this sequence comes from the known homologous sequence from closely related organisms. After synthesis, the nucleic acid sequence can be used alone or joined with a pre-existing sequence and inserted into one of the many available DNA vectors and their respective host cells using techniques well known in the art. Moreover, synthetic chemistry may be used to introduce specific mutations into the nucleotide sequence. Alternatively, a portion of sequence in which a mutation is desired can be synthesized and recombined with a portion of an existing genomic or recombinant sequence.

Hjak2 nucleotide sequence can be used in a diagnostic test or assay to detect disorder or disease processes associated with abnormal expression of hjak2. The nucleotide sequence is added to a sample (fluid, cell or tissue) from a patient under hybridizing conditions. After an incubation period, the sample is washed with a compatible fluid which optionally contains a reporter molecule which will bind the specific nucleotide. After the compatible fluid is rinsed off, the reporter molecule is quantitated and compared with a standard for that fluid, cell or tissue. If hjak2 expression is significantly different from the standard, the assay indicates the presence of disorder or disease. The form of such qualitative or quantitative methods may include northern analysis, dot blot or other membrane-based technologies, dip stick, pin or chip technologies, PCR, ELISAs or other multiple sample format technologies.

This same assay, combining a sample with the nucleotide sequence, is applicable in evaluating the efficacy of a particular therapeutic treatment regime. It may be used in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. First, standard expression must be established for use as a basis of comparison. Second, samples from the animals or patients affected by a disorder or disease are combined with the nucleotide sequence to evaluate the deviation from the standard or normal profile. Third, an entirely new or pre-existing therapeutic agent is administered, and a treatment profile is generated. This posat-treatment assay is evaluated to determine whether the patient profile progresses toward or returns to the standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

The nucleotide sequence for hjak2 can also be used to generate probes for mapping native genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include in situ hybridization to chromosomal spreads (Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of such genetic maps can regularly be found in the journal Science (eg, 1994; 265:1981f). Often the placement of a gene on the chromosome of another mammalian species may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation.

The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. between normal and carrier or affected individuals.

The nucleotide sequence encoding HJAK2 may be used to produce an amino acid sequence using well known methods of recombinant DNA technology. Goeddel (1990, Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego CA) is one among many publications which teach expression of an isolated, purified nucleotide sequence. The amino acid or peptide may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which the nucleotide sequence was derived or from a different species. Advantages of producing an amino acid sequence or peptide by recombinant DNA technology include obtaining adequate amounts for purification and the availability of simplified purification procedures.

Cells transformed with hjak2 nucleotide sequence may be cultured under conditions suitable for the expression and recovery of peptide from cell culture. The peptide produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. In general, it is more convenient to prepare recombinant proteins in secreted form, and this is accomplished by ligating hjak2 to a recombinant nucleotide sequence which directs its movement through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join hjak2 to nucleotide sequence encoding a polypeptide domain which will facilitate protein purification (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

Direct peptide synthesis using solid-phase techniques (Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY pp. 50-60) is an alternative to recombinant or chimeric peptide production. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer in accordance with the instructions provided by the manufacturer. Additionally HJAK2 or any part thereof may be mutated during direct synthesis and combined using chemical methods with other kinase sequences, or parts thereof.

Although an amino acid sequence or oligopeptide used for antibody induction does not require biological activity, it must be immunogenic. HJAK2 used to induce specific antibodies may have an amino acid sequence

consisting of at least five amino acids and preferably at least 10 amino acids. Short stretches of amino acid sequence may be fused with those of another protein such as keyhole limpet hemocyanin, and the chimeric peptide used for antibody production. Alternatively, the peptide may be of sufficient length to contain an entire domain.

Antibodies specific for HJAK2 may be produced by inoculation of an appropriate animal with an antigenic fragment of the peptide. An antibody is specific for HJAK2 if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein. Antibody production includes not only the stimulation of an immune response by injection into animals, but also analogous processes such as the production of synthetic antibodies, the screening of recombinant immunoglobulin libraries for specific-binding molecules (Orlandi R et al (1989) PNAS 86:3833-3837, or Huse WD et al (1989) Science 256:1275-1281), or the <u>in vitro</u> stimulation of lymphocyte populations. Current technology (Winter G and Milstein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules which specifically bind HJAK2. Antibodies or other appropriate molecules generated against a specific immunogenic peptide fragment or oligopeptide can be used in Western analysis, enzyme-linked immunosorbent assays (ELISA) or similar tests to establish the presence of or to quantitate amounts of HJAK2 active in normal, diseased, or therapeutically treated cells or tissues.

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

EXAMPLES

I Placenta cDNA Library Construction

Library was constructed from normal placenta obtained from the Mayo Clinic. The tissue was lysed in a buffer containing guanidinium isothiocyanate. The lysate was extracted with phenol chloroform and precipitated with ethanol. Poly A$^+$ RNA was isolated using biotinylated oligo d(T) primer and steptavidin coupled to a paramagnetic particle

(Promega Corp. Madison WI) and sent to Stratagene (La Jolla CA) for cDNA library preparation. The cDNA synthesis was primed using both oligo d(T) and random hexamers, and the two cDNA libraries were treated separately. Synthetic adapter oligonucleotides were ligated onto the ends of the cDNAs which were digested with XhoI and inserted into the Uni-ZAP™ vector system (Stratagene).

The pBluescript™ phagemid (Stratagene) was excised from each library , and phagemids from the two cDNA libraries were combined into a single library by mixing equal numbers of bacteriophage. The phagemids were transformed into E. coli host strain XL1-Blue™ (Stratagene). Enzymes from both pBluescript and a cotransformed f1 helper phage nicked the DNA, initiated new DNA synthesis, and created the smaller, single-stranded circular plasmid DNA molecules which contained the cDNA insert. The plasmid DNA was released, purified, and used to reinfect fresh host cells (SOLR, Stratagene). Presence of the ß-lactamase gene on the plasmid allowed transformed bacteria to grow on medium containing ampicillin.

II Isolation of cDNA Clones

Plasmid DNAs containing the cDNA insert were purified using the QIAWELL-8 Plasmid Purification System from QIAGEN Inc (Chatsworth CA) according to standard protocol. The DNA was eluted and prepared for DNA sequencing and other analytical manipulations.

The cDNA inserts from random isolates of the placenta library were partially sequenced. The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Catalyst 800 or a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer), and reading frame was determined.

III Sequencing of cDNA Clones

The cDNA inserts from random isolates of the placenta library were sequenced in part. Methods for DNA sequencing are well known in the art and employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE® (US Biochemical Corp) or Taq polymerase. Methods to extend the DNA from an oligonucleotide primer annealed to the DNA template of interest have been developed for both single- and double-stranded templates. Chain

termination reaction products were separated using electrophoresis and detected via their incorporated, labelled precursors. Recent improvements in mechanized reaction preparation, sequencing and analysis have permitted expansion in the number of sequences that can be determined per day. Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the Applied Biosystems Catalyst 800 and 377 and 373 DNA sequencers.

The quality of any particular cDNA library may be determined by performing a pilot scale analysis of the cDNAs and checking for percentages of clones containing vector, lambda or E. coli DNA, mitochondrial or repetitive DNA, and clones with exact or homologous matches to public databases. The number of unique sequences, those having no known match in any available database, are then recorded.

IV Homology Searching of cDNA Clones and Their Deduced Proteins

Each sequence so obtained was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT™ 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search

Tool, is used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. While it is useful for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

The partial hjak2 molecule presented and claimed in this application was identified using the criteria above. The full length nucleic and amino acid sequences for this novel human Jak2 kinase are shown in Fig 1. Fig 2 shows the alignment between the translated amino acid sequence for hjak2 and the closest related molecule, murine Jak2 kinase (MUSPTK1; GenBank GI 409584; Wilks AF (1989) Proc Nat Acad Sci 86:1603-7).

V Extension of cDNAs to Full Length

The partial sequence originally identified in Incyte Clone 179527 was used to design oligonucleotide primers for extension of the cDNAs to full length. Primers are designed based on known sequence; one primer is synthesized to initiate extension in the antisense direction (XLR) and the other to extend sequence in the sense direction (XLF). The primers allow the sequence to be extended "outward" generating amplicons containing new, unknown nucleotide sequence for the gene of interest. The primers may be designed using Oligo 4.0 (National Biosciences Inc, Plymouth MN), or

another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

The placenta cDNA library was used with XLR = GGGCGGAAGTGCTCTCGGCGGAAG and XLF = AGTGTGCTACAGTGCTGGTCGTCG primers to extend and amplify Incyte Clone 179527 to obtain the full length Jak2 kinase sequence.

By following the instructions for the XL-PCR kit and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5-10 μl aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Although all extensions potentially contain a full length gene, some of the largest products or bands are selected and cut out of the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μl of ligation buffer. Then, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent E. coli cells

(in 40 μl of appropriate media) are transformed with 3 μl of ligation mixture and cultured in 80 μl of SOC medium (Sambrook J et al, supra). After incubation for one hour at 37° C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook J et al, supra) containing 2xCarb. The following day, 12 colonies are randomly picked from each plate and cultured in 150 μl of liquid LB/2xCarb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μl of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μl of each sample is transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2-4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid and sequenced.

VI Diagnostic Assay Using Hjak2 Specific Oligomers

In those cases where a specific condition (see definitions, supra) is suspected to involve expression of altered quantities of hjak2, oligomers may be designed to establish the presence and/or quantity of mRNA expressed in a biological sample. There are several methods currently being used to quantitate the expression of a particular molecule. Most of these methods use radiolabelled (Melby PC et al 1993 J Immunol Methods 159:235-44) or biotinylated (Duplaa C et al 1993 Anal Biochem 229-36) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation may be speeded up by running the assay in an ELISA format where the oligomer-of-interest is presented in various dilutions and a colorimetric response gives rapid

quantitation. For example, a complete HJAK2 deficiency may result in the inability to undergo cell division or to react to an infectious organism. In like manner, overexpression may cause major inflammation, swelling and major tissue damage. In either case, a quick diagnosis may allow health professionals to treat the condition and prevent worsening of the condition. This same assay can be used to monitor progress of the patient as his/her physiological situation moves toward the normal range during therapy.

VII Sense or Antisense Molecules

Knowledge of the correct cDNA sequence of this Jak2 kinase or its regulatory elements enable its use as a tool in sense (Youssoufian H and HF Lodish 1993) Mol Cell Biol 13:98-104) or antisense (Eguchi et al (1991) Annu Rev Biochem 60:631-652) technologies for the investigation or alteration of gene expression. To inhibit in vivo or in vitro cdp expression, an oligonucleotide based on the coding sequence of an hjak2 designed with Oligo 4.0 (National Biosciences Inc) is used. Alternatively, a fragment of an hjak2 is produced by digesting hjak2 coding sequence with restriction enzymes. These enzymes and specific restrictions sites may be selected using Inherit Analysis software (Applied Biosystems), and the strands separated by heating the fragments and selecting for the antisense strand. Either the oligonucleotide or the fragment may be used to inhibit hjak2 expression. Furthermore, antisense molecules can be designed to inhibit promoter binding in the upstream nontranslated leader or at various sites along the hjak2 coding region. Alternatively, antisense molecules may be designed to inhibit translation of an mRNA into polypeptide by preparing an oligomer or fragment which will bind in the region spanning approximately -10 to +10 nucleotides at the 5' end of the coding sequence. These technologies are now well known to those of in the art.

In addition to using antisense molecules constructed to interrupt transcription of the open reading frame, modifications of gene expression can be obtained by designing antisense sequences to enhancers, introns, or even to trans-acting regulatory genes. Similarly, inhibition can be achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases,

transcription factors, or regulatory molecules.

Any of these types of antisense molecules may be placed in expression vectors and used to transform preferred cells or tissues. This may include introduction of the expression vector into a organ, tumor, synovial cavity or the vascular system for transient or short term therapy or introduction via gene therapy technologies for long term treatment. Transient expression may last for a month or more with a non-replicating vector and three months or more if appropriate replication elements are used in the transformation or expression system.

Stable transformation of appropriate dividing cells with a vector containing the antisense molecule can produce a transgenic cell line, tissue or organism (see, for example, Trends in Biotechnol 11:155-215 (1993) and US Patent No. 4,736,866, 12 April 1988). Those cells which assimilate or replicate enough copies of the vector to allow stable integration will also produce enough antisense molecules to compromise or entirely eliminate normal activity of the hjak2. Frequently, the function of an hjak2 can be ascertained by observing behaviors such as lethality, loss of a physiological pathway, changes in morphology, etc. at the cellular, tissue or organismal level.

VIII Expression of HJAK2

Expression of the HJAK2 may be accomplished by subcloning the cDNA into appropriate vectors and transfecting the vectors into host cells. In this case, the cloning vector previously used for the generation of the tissue library also provides for direct expression of the hjak2 sequence in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transfected bacterial strain with IPTG using standard methods will produce a fusion protein corresponding to the first seven residues of ß-galactosidase, about 5 to 15 residues which correspond to linker, and the peptide encoded within the hjak2 cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it

can be obtained by deletion or insertion of the appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide linkers containing cloning sites as well as a stretch of DNA sufficient to hybridize to the end of the target cDNA (25 bases) can be synthesized chemically by standard methods. These primers can then used to amplify the desired gene fragments by PCR. The resulting fragments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternatively, similar gene fragments can be produced by digestion of the cDNA with appropriate restriction enzymes and filling in the missing gene sequence with chemically synthesized oligonucleotides. Partial nucleotide sequence from more than one kinase homolog can be ligated together and cloned into appropriate vectors to optimize expression.

Suitable expression hosts for such chimeric molecules include but are not limited to mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the ß-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

If native promoters are not part of the cDNA, other host specific promoters may be specifically combined with the coding region of hjak2. They include MMTV, SV40, and metallothionine promoters for CHO cells; trp, lac, tac and T7 promoters for bacterial hosts; and alpha factor, alcohol oxidase and PGH promoters for yeast. In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained

through standard culture methods, large quantities of recombinantly produced peptide can be recovered from the conditioned medium and analyzed using methods known in the art.

IX Isolation of Recombinant HJAK2

HJAK2 may be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine- tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen) between the purification domain and the hjak2 sequence may be useful to facilitate expression of HJAK2.

X Testing HJAK2 Activity

The sequence for HJAK2 in this application present many different domains (and subdomains as detailed in the background of the invention) which may be utilized: 1) individually for the production of antibodies, 2) in functional groups (eg. to span a membrane), and 3) as interchangable, usable parts of a chimeric kinase. For example, a known, full length kinase such as the hjak2 kinase of this application may be used to swap related portions of the nucleic acid sequence, analogous to domains or subdomains of MAP kinase polypeptides. The chimeric nucleotides, so produced, may be introduced into prokaryotic host cells (as reviewed in Strosberg AD and Marullo S (1992) Trends Pharma Sci 13:95-98) or eukaryotic host cells. These host cells are then employed in procedures to determine what molecules activate the kinase or what molecules are activated by a kinase. Such activating or activated molecules may be of extracellular, intracellular, biologic or chemical origin.

An example of a test system, in this case for hjak2 kinase, can be based on the interaction of protein tyrosine kinases with chemokine receptors (Taniguchi T (1995) Science 268:251-255). These receptors are capable of activating a variety of nonreceptor protein tyrosine kinases when stimulated by an extracellular chemokine. C-X-C chemokines such as

platelet factor 4, interleukin-8, connective tissue activating protein III, neutrophil activating peptide 2, are soluble activators of neutrophils.

A standard measure of neutrophil activation involves measuring the mobilization of Ca^{++} as part of the signal transduction pathway. The experiment involves several steps. First, blood cells obtained from venipuncture are fractionated by centrifugation on density gradients. Enriched populations of neutrophils are further fractionated on columns by negative selection using antibodies specific for other blood cells types. Next, neutrophils are transformed with an expression vector containing the kinase nucleic acid sequence of interest and preloaded fluorescent probe whose emission characteristics have been altered by Ca^{++} binding. Or in the alternative, the neutrophil is preloaded with the purified kinase of interest and fluorescent probe. Then, when the cells are exposed to an appropriate chemokine, the chemokine receptor activates the kinase which, in turn, initiates Ca^{++} flux. Ca^{++} mobilization is observed and measured using fluorometry as has been described in Grynkievicz G et al (1985) J Biol Chem 260:3440, and McColl S et al (1993) J Immunol 150:4550-4555, incorporated herein by reference.

XI Identification of or Production of HJAK2 Specific Antibodies

Purified HJAK2 is used to screen a pre-existing antibody library or to raise antibodies using either polyclonal or monoclonal methodology. In a polyclonal approach, denatured protein from the reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein can be used to immunize mice or rabbits using standard protocols; about 100 micrograms are adequate for immunization of a mouse, while up to 1 mg might be used to immunize a rabbit. For identifying mouse hybridomas, the denatured protein can be radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg would be sufficient for labeling and screening of several thousand clones.

In a monoclonal approach, the amino acid sequence of HJAK2, as deduced from translation of the cDNA, is analyzed to determine regions of high immunogenicity. Oligopeptides comprising appropriate hydrophilic regions, as shown in Fig. 3, are synthesized and used in suitable immunization protocols to raise antibodies. Analysis to select appropriate epitopes is described by Ausubel FM et al (supra). The optimal amino acid

sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl- N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine may be introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% BSA, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas may also be prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled HJAK2 to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated with affinity purified, specific rabbit-anti-mouse antibodies (or suitable anti-species Ig) at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and exposed to supernatants from hybridomas. After incubation the wells are exposed to labeled HJAK2, 1 mg/ml. Clones producing antibodies will bind a quantity of labeled HJAK2 which is detectable above background. Such clones are expanded and subjected to 2 cycles of cloning at limiting dilution (1 cell/3 wells). Cloned hybridomas are injected into pristine mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least 10^8 /M, preferably 10^9 to 10^{10} or stronger, will typically be made by standard procedures as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; and in Goding (1986) Monoclonal Antibodies: Principles and Practice, Academic Press, New York City, both incorporated herein by reference.

XII Diagnostic Test Using HJAK2 Specific Antibodies

Particular HJAK2 antibodies are useful for the diagnosis of prepathologic conditions, and chronic or acute diseases which are characterized by differences in the amount or distribution of HJAK2. To date, HJAK2 has been found only in the placenta library; however, its activity there is most probably associated with organ function, inflammation or defense.

Diagnostic tests for HJAK2 include methods utilizing the antibody and a label to detect HJAK2 in human body fluids, tissues or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemi-luminescent, or chromogenic agents previously mentioned as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567, incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound HJAK2, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HJAK2 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

XIII Purification of Native HJAK2 Using Specific Antibodies

Native or recombinant HJAK2 can be purified by immunoaffinity chromatography using antibodies specific for that particular HJAK2. In general, an immunoaffinity column is constructed by covalently coupling the

anti-HJAK2 antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia Biotech). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia Biotech). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such immunoaffinity columns may be utilized in the purification of HJAK2 by preparing a fraction from cells containing HJAK2 in a soluble form. This preparation may be derived by solubilization of whole cells or of a subcellular fraction obtained via differential centrifugation (with or without addition of detergent) or by other methods well known in the art. Alternatively, soluble HJAK2 containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble HJAK2-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HJAK2 (eg, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/HJAK2 binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and HJAK2 is collected.

XIV Drug Screening

This invention is particularly useful for screening therapeutic compounds by using binding fragments of HJAK2 in any of a variety of drug screening techniques. The peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One may measure, for example, the formation of complexes between HJAK2 and the agent being tested. Alternatively, one can examine the diminution in complex formation between HJAK2 and a receptor caused by the agent being tested.

Methods of screening for drugs or any other agents which can affect macrophage activation comprise contacting such an agent with HJAK2 fragment and assaying for the presence of a complex between the agent and the HJAK2

fragment. In such assays, the HJAK2 fragment is typically labelled. After suitable incubation, free HJAK2 fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to HJAK2.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the HJAK2 polypeptides and is described in detail in European Patent Application 84/03564, published on September 13, 1984, incorporated herein by reference. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with HJAK2 fragment and washed. Bound HJAK2 fragment is then detected by methods well known in the art. Purified HJAK2 can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding HJAK2 specifically compete with a test compound for binding to HJAK2 fragments. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HJAK2.

XV Identification of Molecules Which Interact with HJAK2

The inventive purified HJAK2 is a research tool for identification, characterization and purification of interacting molecules. Appropriate labels are incorporated into HJAK2 by various methods known in the art and HJAK2 is used to capture soluble or interact with membrane-bound molecules. A preferred method involves labeling the primary amino groups in HJAK2 with ^{125}I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J 133: 529). This reagent has been used to label various molecules without concomitant loss of biological activity (Hebert CA et al (1991) J Biol Chem 266: 18989-94; McColl S et al (1993) J Immunol 150:4550-4555). Membrane-bound molecules are incubated with the labelled HJAK2 molecules, washed to removed unbound molecules, and the HJAK2 complex is quantified. Data obtained using different concentrations of HJAK2 are used to calculate values for the number, affinity, and association of HJAK2.

Labelled HJAK2 fragments are also useful as a reagent for the

purification of molecules with which HJAK2 interacts, specifically including inhibitors. In one embodiment of affinity purification, HJAK2 is covalently coupled to a chromatography column. Cells and their membranes are extracted, HJAK2 is removed and various HJAK2-free subcomponents are passed over the column. Molecules bind to the column by virtue of their HJAK2 affinity. The HJAK2-complex is recovered from the column, dissociated and the recovered molecule is subjected to N-terminal protein sequencing or other identification procedure. If the captured molecule has an amino acid sequence, it can be used to design degenerate oligomers for use in cloning the gene from an appropriate cDNA library.

In an alternate method, monoclonal antibodies raised against HJAK2 fragments are screened to identify those which inhibit the binding of labelled HJAK2. These monoclonal antibodies are then used in affinity purification or expression cloning of associated molecules. Other soluble binding molecules are identified in a similar manner. Labelled HJAK2 is incubated with extracts or other appropriate materials derived from lung, kidney or other tissues with activated monocytes or macrophages. After incubation, HJAK2 complexes (which are larger than the lone HJAK2 fragment) are identified by a sizing technique such as size exclusion chromatography or density gradient centrifugation and are purified by methods well known in the art. The soluble binding protein(s) are subjected to N-terminal sequencing to obtain information sufficient for database identification, if the soluble protein is known, or for cloning, if the soluble protein is unknown.

XVI Use and Administration of Antibodies or Inhibitors to HJAK2

The antibodies and inhibitors can provide different effects when administered therapeutically. The antibodies and inhibitors are used to lessen or eliminate undue damage caused by disorders or diseases associated with upregulated HJAK2 expression. Each of these molecules or treatments (TSTs) will be formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although the pH may vary according to the different characteristics of the peptide, antibody or inhibitor being formulated and the condition to be treated. Characteristics of TSTs include solubility of the molecule, half-life, antigenicity/immunogenicity and the ability of the inhibitor to reach its target(s). These and other characteristics may aid in defining

an effective carrier. Native human proteins are preferred as TSTs, but recombinant peptides as well as organic or synthetic molecules resulting from drug screens may be equally effective in particular situations.

TSTs may be delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol; transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration will be determined by the attending physician and will vary according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the TST to be administered, and the pharmacokinetic profile of the particular TST. Additional factors which may be taken into account include disease state (eg. severity) of the patient, age, weight, gender, diet, time and frequency of administration, drug combination, reaction sensitivities, and tolerance/response to therapy. Long acting TST formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular TST.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature. See US Patent No. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art will employ different formulations for different TSTs. Administration to lung cells may necessitate delivery in a manner different from that to kidney or other cells.

It is contemplated that conditions associated with altered HJAK2 expression are treatable with TSTs. These conditions, which specifically include, but are not limited to, anemia, arteriosclerosis, asthma, bronchitis, emphysema, gingivitis, inflammatory bowel disease, insulin-dependent diabetes mellitus, leukemia, multiple endocrine neoplasias, osteoarthritis, osteoporosis, pulmonary fibrosis, rheumatoid arthritis, septic shock syndromes, and systemic lupus erythematosus may be specifically diagnosed by the tests discussed above. In addition, such tests may be used to monitor treatment.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

PF-0049 US

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Coleman, Roger
Stuart, Susan G.

(ii) TITLE OF INVENTION: A NOVEL HUMAN JAK2 KINASE HOMOLOG

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
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(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Luther, Barbara J.
(B) REGISTRATION NUMBER: 33954
(C) REFERENCE/DOCKET NUMBER: PF-0049 US

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-855-0555
(B) TELEFAX: 415-852-0195

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4482 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Placenta
(B) CLONE: 179527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCACGCGTCC GGTTGCCAAC CCGCAGGCGA CTGGGCGCTT CATCCCACCC TCACCCCTTT 60

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GGGAAGGACG GGTCTGTTGT ACCCGGGAGG TGGAAGGAAA AGCCGAAAGC GGAGAAGTGT 240

GCGGGAGGGG AGTCTCCGCG CGGAGGNAGA CCGGNCTCCT CCAGTGCAGG TTGTGCGCTG 300

GGGAGCCAGC CASGGCAAAT GTTCTGAAAA AGACTCTGCA TGGGAATGGC CTGCCTTACG 360

ATGACAGAAA TGGAGGGAAC ATCCACCTCT TCTATATATC AGAATGGTGA TATTTCTGGA 420

AATGCCAATT CTATGAAGCA AATAGATCCA GTTCTTCAGG TGTATCTTTA CCATTCCCTT 480

GGGAAATCTG AGGCAGATTA TCTGACCTTT CCATCTGGGG AGTATGTTGG AGAAGAAATC 540

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ACCAGGCATA ATGTACTCTA CAGAATAAGA TTTTACTTTC CTCGTTGGTA TTGCAGTGGC 720

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CGAATAAGGT ACAGATTTCG CAGATTTATT CAGCAATTCA GCCAATGCAA AGCCACTGCC 1080

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TTAATTGATG GATATTATAG ATTAACTGCA GATGCACATC ATTACCTCTG TAAAGAAGTA 1500

GCACCTCCAG CCGTGCTTGA AAATATACAA AGCAACTGTC ATGGCCCAAT TTCGATGGAT 1560

TTTGCCATTA GTAAACTGAA GAAAGCAGGT AATCAGACTG GACTGTATGT ACTTCGATGC 1620

AGTCCTAAGG ACTTTAATAA ATATTTTTTG ACTTTTGCTG TCGAGCGAGA AAATGTCATT 1680

GAATATAAAC ACTGTTTGAT TACAAAAAAT GAGAATGAAG AGTACAACCT CAGTGGGACA 1740

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ACCCTTATTC ATGGGAATGT ATGTGCCAAA AATATTCTGC TTATCAGAGA AGAAGACAGG 2400

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CATGATGCTA GCCAGCAAAG ATGTGAAAAT ATCTGCTCAA AACTTTCAAA GTTTAGTAAG 3900

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TAAGAAGTTT CTTAAACATT GTCAGTTAAC ATCACTCTTG TCTGGCAAAA GAAAAAAAAT 4020

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ATTGAATAAG TACCTTTGTG TCCTTGTTCA TTTATATCGC TGGCCAGCAT TATAAGCAGG 4320

TGTATACTTT TAGCTTGTAG TTCCATGTAC TGTAAATATT TTTCACATAA AGGGAACAAA 4380

TGTCTAGTTT TATTTGTATA GGAAATTTGC CCTGACCCTA AATAATACAT TTTGAAATGA 4440

AACAAGCTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AG 4482

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1132 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr
 1               5                  10                  15

Ser Ser Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met
            20                  25                  30

Lys Gln Ile Asp Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly
        35                  40                  45

Lys Ser Glu Ala Asp Tyr Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly
    50                  55                  60

Glu Glu Ile Cys Ile Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65                  70                  75                  80

Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
                85                  90                  95

Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asn Val
            100                 105                 110

Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser Gly Ser
        115                 120                 125

Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu
    130                 135                 140

Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp
145                 150                 155                 160
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Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
                165                 170                 175

Glu Cys Leu Gly Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu
            180                 185                 190

Asn Asp Gln Thr Pro Leu Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr
        195                 200                 205

Phe Leu Pro Gln Cys Ile Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
    210                 215                 220

Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225                 230                 235                 240

Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu Lys Tyr Leu Ile
                245                 250                 255

Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe Glu Val
            260                 265                 270

Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
        275                 280                 285

Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys
    290                 295                 300

Glu Ser Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe
305                 310                 315                 320

Pro Asn Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser
                325                 330                 335

Asn Glu Ser Arg Val Val Thr Ile His Lys Gln Asp Gly Lys Asn Leu
            340                 345                 350

Glu Ile Glu Leu Ser Ser Leu Arg Glu Ala Leu Ser Phe Val Ser Leu
        355                 360                 365

Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp Ala His His Tyr Leu Cys
    370                 375                 380

Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile Gln Ser Asn Cys
385                 390                 395                 400

His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys Lys Ala
                405                 410                 415

Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
            420                 425                 430
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Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu
435 440 445

Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu
450 455 460

Ser Gly Thr Lys Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys
465 470 475 480

Tyr Gln Met Glu Thr Val Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr
485 490 495

Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
500 505 510

Arg Thr Asn Gly Val Ser Asp Val Pro Thr Ser Pro Thr Leu Gln Arg
515 520 525

Pro Thr His Met Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
530 535 540

Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys Ile Phe
545 550 555 560

Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr
565 570 575

Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu
580 585 590

Ser Phe Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His
595 600 605

Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu
610 615 620

Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
625 630 635 640

Asn Lys Asn Cys Ile Asn Ile Leu Trp Lys Leu Glu Val Ala Lys Gln
645 650 655

Leu Ala Trp Ala Met His Phe Leu Glu Glu Asn Thr Leu Ile His Gly
660 665 670

Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Lys
675 680 685

Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
690 695 700

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Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
705                 710                 715                 720

Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
                725                 730                 735

Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
            740                 745                 750

Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
        755                 760                 765

Arg His Gln Leu Pro Ala Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile
    770                 775                 780

Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ser Phe Arg Ala
785                 790                 795                 800

Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
                805                 810                 815

Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
            820                 825                 830

Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
        835                 840                 845

Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
    850                 855                 860

Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
865                 870                 875                 880

Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
                885                 890                 895

Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
            900                 905                 910

Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile
        915                 920                 925

Met Glu Tyr Leu Pro Tyr Gly Ser Leu Arg Asp Tyr Leu Gln Lys His
    930                 935                 940

Lys Glu Arg Ile Asp His Ile Lys Leu Leu Gln Tyr Thr Ser Gln Ile
945                 950                 955                 960

Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
                965                 970                 975
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Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
            980                 985                 990

Gly Ile Leu Gly  *  Pro Lys Ser Cys His Lys Thr Lys Asn Thr Ile
        995                1000                1005

Lys  *  Lys Asn Leu Val Lys Val Pro Tyr Ser Gly Met Leu Gln Asn
    1010                1015                1020

His  *  Gln Arg Ala Ser Phe Leu Trp Pro Gln Met Phe Gly Ala Leu
1025                1030                1035                1040

Glu Trp Phe Cys Met Asn Phe Ser His Thr Leu Arg Arg Val Lys Val
                1045                1050                1055

His Gln Arg Asn Leu Cys Val  *  Leu Ala Met Thr Asn Lys Asp Arg
            1060                1065                1070

 *  Ser Cys Ser Ile  *   *  Asn Phe  *  Arg Ile Met Glu Asp Tyr
        1075                1080                1085

Gln Asp Gln Met Asp Ala Gln Met Arg Ser Ile  *  Ser  *  Gln Asn
    1090                1095                1100

Ala Gly Thr Ile Met  *  Ile Asn Ala Pro Pro Leu Gly Ile  *  Leu
1105                1110                1115                1120

Phe Glu Trp Ile Lys  *  Gly Ile Thr Trp Leu Asp
                1125                1130
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CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide of SEQ ID NO:2, or the complement of said polynucleotide.

2. The polynucleotide of Claim 1 comprising the nucleic acid sequence for a novel human Jak2 kinase (hjak2) of SEQ ID NO:1.

3. An antisense molecule comprising the complement of the polynucleotide of Claim 2 or a portion thereof.

4. A pharmaceutical composition comprising the antisense molecule of Claim 3 and a pharmaceutically acceptable excipient.

5. A method of treating a subject with a condition associated with altered hjak2 expression comprising administering an effective amount of the pharmaceutical composition of Claim 4 to the subject.

6. A diagnostic composition comprising an oligomer of the polynucleotide of Claim 2.

7. A diagnostic test for a condition associated with altered hjak2 expression comprising the steps of:

a) providing a biological sample;

b) combining the biological sample and the diagnostic composition of Claim 6;

c) allowing hybridization to occur between the biological sample and the diagnostic composition under suitable conditions;

d) measuring the amount of hybridization to obtain a sample value; and

e) comparing the sample value with standard values to determine whether hjak2 expression is altered.

8. An expression vector comprising the polynucleotide of Claim 1.

9. A host cell transformed with the expression vector of Claim 8.

10. A method for producing a polypeptide, said method comprising the steps of:

a) culturing the host cell of Claim 9 under conditions suitable for the expression of

the polypeptide; and

b) recovering the polypeptide from the host cell culture.

11. A purified polypeptide (HJAK2) comprising the amino acid sequence of SEQ ID NO:2.

12. A diagnostic composition comprising the polypeptide of Claim 11 or a portion thereof.

13. A pharmaceutical composition comprising the polypeptide of Claim 11 and a pharmaceutically acceptable excipient.

14. A method of treating a subject with a condition associated with altered HJAK2 expression comprising administering an effective amount of the pharmaceutical composition of Claim 13 to the subject.

15. An antibody specific for the purified polypeptide of Claim 11, or portion thereof.

16. A diagnostic composition comprising the antibody of Claim 15.

17. A diagnostic test for a condition associated with altered HJAK2 expression comprising the steps of:

a) providing a biological sample;

b) combining the biological sample and the antibody of Claim 15 under conditions suitable for complex formation;

c) measuring the amount of complex formation between HJAK2 and the antibody to obtain a sample amount; and

d) comparing the amount of complex formation in the sample with standard amounts of complex formation, wherein a variation between the sample amount and standard amounts of complex formation establishes the presence of the condition.

18. A method of screening a plurality of compounds for specific binding affinity with the polypeptide of Claim 11 or any portion thereof comprising the steps of:

a) providing a plurality of compounds;

b) combining HJAK2 with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and

c) detecting binding of HJAK2 to each of the plurality of compounds, thereby identifying the compounds which specifically bind HJAK2.

ABSTRACT

A NOVEL HUMAN JAK2 KINASE

The present invention provides a polynucleotide (hjak2) which identifies and encodes a novel human Jak2 kinase (HJAK2) which was expressed in the placenta. The present invention also provides for antisense molecules and oligomers designed from the nucleotide sequence or its antisense. The invention further provides genetically engineered expression vectors and host cells for the production of purified HJAK2 peptide, antibodies capable of binding to HJAK2, inhibitors which bind to HJAK2 and pharmaceutical compositions based on HJAK2 specific antibodies or inhibitors. The invention specifically provides for diagnostic assays based on altered hjak2 expression and which allow identification of such a condition. These assays utilize probes which comprise oligomers, fragments, or portions of hjak2 or its regulatory elements or antibodies specifically binding HJAK2.

"Express Mail" mailing label number EL 315 815 124 US. I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Assistant Commissioner for Patents, Box PATENT APPLICATION, Washington, D.C. 20231 on 12-10-99

By: Nancy Ramos

Printed: Nancy Ramos

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: A NOVEL HUMAN JAK2 KINASE

Serial No.:	To Be Assigned	Filing Date:	Herewith
Examiner:	To Be Assigned	Group Art Unit:	To Be Assigned

Official Draftsman
Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

SUBMISSION OF FORMAL DRAWINGS

Sir:

Transmitted herewith are Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 2A, 2B, 2C, 2D, 2E, as thirteen (13) sheets of formal drawings for this application. Each sheet of drawing indicates the identifying indicia suggested in 37 CFR Section 1.84(c) on the reverse side of the drawings.

Applicants believe that no fee is due with this paper. However, if the Commissioner determines that a fee is necessary, the Commissioner is hereby authorized to charge any additional fees associated with this communication or credit any overpayment to Incyte Pharmaceuticals, Inc. Deposit Account No. **09-0108. A duplicate copy of this communication is enclosed.**

If there are any questions regarding the above, the Examiner is invited to call the undersigned at 650-855-0555.

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Date: December 9, 1999

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Fax: (650) 849-8886

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            9          18          27          36          45          54
5' ATG GGA ATG GCC TGC CTT ACG ATG ACA GAA ATG GAG GGA ACA TCC ACC TCT TCT
   Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr Ser Ser

           63          72          81          90          99         108
   ATA TAT CAG AAT GGT GAT ATT TCT GGA AAT GCC AAT TCT ATG AAG CAA ATA GAT
   Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met Lys Gln Ile Asp

          117         126         135         144         153         162
   CCA GTT CTT CAG GTG TAT CTT TAC CAT TCC CTT GGG AAA TCT GAG GCA GAT TAT
   Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly Lys Ser Glu Ala Asp Tyr

          171         180         189         198         207         216
   CTG ACC TTT CCA TCT GGG GAG TAT GTT GGA GAA GAA ATC TGT ATT GCT GCT TCT
   Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly Glu Glu Ile Cys Ile Ala Ala Ser

          225         234         243         252         261         270
   AAA GCT TGT GGT ATC ACA CCT GTG TAT CAT AAT ATG TTT GCT TTA ATG AGT GAA
   Lys Ala Cys Gly Ile Thr Pro Val Tyr His Asn Met Phe Ala Leu Met Ser Glu

          279         288         297         306         315         324
   ACA GAA AGG ATC TGG TAT CCA CCC AAC CAT GTC TTC CAT ATA GAT GAG TCA ACC
   Thr Glu Arg Ile Trp Tyr Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr

          333         342         351         360         369         378
   AGG CAT AAT GTA CTC TAC AGA ATA AGA TTT TAC TTT CCT CGT TGG TAT TGC AGT
   Arg His Asn Val Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser

          387         396         405         414         423         432
   GGC AGC AAC AGA GCC TAT CGG CAT GGA ATA TCT CGA GGT GCT GAA GCT CCT CTT
   Gly Ser Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu

          441         450         459         468         477         486
   CTT GAT GAC TTT GTC ATG TCT TAC CTC TTT GCT CAG TGG CGG CAT GAT TTT GTG
   Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp Phe Val
```

FIGURE 1A

```
        495         504         513         522         531         540
CAT GGA TGG ATA AAA GTA CCT GTG ACT CAT GAA ACA CAG GAA GAA TGT CTT GGG
His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu Glu Cys Leu Gly

        549         558         567         576         585         594
ATG ACA GTG TTA GAT ATG ATG AGA ATA GCC AAA GAA AAC GAT CAA ACC CCA CTG
Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu Asn Asp Gln Thr Pro Leu

        603         612         621         630         639         648
GCC ATC TAT AAC TCT ATC AGC TAC AAG ACA TTC TTA CCA CAA TGT ATT CGA GCA
Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr Phe Leu Pro Gln Cys Ile Arg Ala

        657         666         675         684         693         702
AAG ATC CAA GAC TAT CAT ATT TTG ACA AGG AAG CGA ATA AGG TAC AGA TTT CGC
Lys Ile Gln Asp Tyr His Ile Leu Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg

        711         720         729         738         747         756
AGA TTT ATT CAG CAA TTC AGC CAA TGC AAA GCC ACT GCC AGA AAC TTG AAA CTT
Arg Phe Ile Gln Gln Phe Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu

        765         774         783         792         801         810
AAG TAT CTT ATA AAT CTG GAA ACT CTG CAG TCT GCC TTC TAC ACA GAG AAA TTT
Lys Tyr Leu Ile Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe

        819         828         837         846         855         864
GAA GTA AAA GAA CCT GGA AGT GGT CCT TCA GGT GAG GAG ATT TTT GCA ACC ATT
Glu Val Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile

        873         882         891         900         909         918
ATA ATA ACT GGA AAC GGT GGA ATT CAG TGG TCA AGA GGG AAA CAT AAA GAA AGT
Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys Glu Ser

        927         936         945         954         963         972
GAG ACA CTG ACA GAA CAG GAT TTA CAG TTA TAT TGC GAT TTT CCT AAT ATT ATT
Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe Pro Asn Ile Ile
```

FIGURE 1B

```
         981         990         999        1008        1017        1026
GAT GTC AGT ATT AAG CAA GCA AAC CAA GAG GGT TCA AAT GAA AGC CGA GTT GTA
Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser Asn Glu Ser Arg Val Val

        1035        1044        1053        1062        1071        1080
ACT ATC CAT AAG CAA GAT GGT AAA AAT CTG GAA ATT GAA CTT AGC TCA TTA AGG
Thr Ile His Lys Gln Asp Gly Lys Asn Leu Glu Ile Glu Leu Ser Ser Leu Arg

        1089        1098        1107        1116        1125        1134
GAA GCT TTG TCT TTC GTG TCA TTA ATT GAT GGA TAT TAT AGA TTA ACT GCA GAT
Glu Ala Leu Ser Phe Val Ser Leu Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp

        1143        1152        1161        1170        1179        1188
GCA CAT CAT TAC CTC TGT AAA GAA GTA GCA CCT CCA GCC GTG CTT GAA AAT ATA
Ala His His Tyr Leu Cys Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile

        1197        1206        1215        1224        1233        1242
CAA AGC AAC TGT CAT GGC CCA ATT TCG ATG GAT TTT GCC ATT AGT AAA CTG AAG
Gln Ser Asn Cys His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys

        1251        1260        1269        1278        1287        1296
AAA GCA GGT AAT CAG ACT GGA CTG TAT GTA CTT CGA TGC AGT CCT AAG GAC TTT
Lys Ala Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe

        1305        1314        1323        1332        1341        1350
AAT AAA TAT TTT TTG ACT TTT GCT GTC GAG CGA GAA AAT GTC ATT GAA TAT AAA
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu Tyr Lys

        1359        1368        1377        1386        1395        1404
CAC TGT TTG ATT ACA AAA AAT GAG AAT GAA GAG TAC AAC CTC AGT GGG ACA AAG
His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu Ser Gly Thr Lys
```

FIGURE 1C

```
   1413        1422        1431        1440        1449        1458
AAG AAC TTC AGC AGT CTT AAA GAT CTT TTG AAT TGT TAC CAG ATG GAA ACT GTT
Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys Tyr Gln Met Glu Thr Val

    1467        1476        1485        1494        1503        1512
CGC TCA GAC AAT ATA ATT TTC CAG TTT ACT AAA TGC TGT CCC CCA AAG CCA AAA
Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr Lys Cys Cys Pro Pro Lys Pro Lys

       1521        1530        1539        1548        1557        1566
GAT AAA TCA AAC CTT CTA GTC TTC AGA ACG AAT GGT GTT TCT GAT GTA CCA ACC
Asp Lys Ser Asn Leu Leu Val Phe Arg Thr Asn Gly Val Ser Asp Val Pro Thr

       1575        1584        1593        1602        1611        1620
TCA CCA ACA TTA CAG AGG CCT ACT CAT ATG AAC CAA ATG GTG TTT CAC AAA ATC
Ser Pro Thr Leu Gln Arg Pro Thr His Met Asn Gln Met Val Phe His Lys Ile

       1629        1638        1647        1656        1665        1674
AGA AAT GAA GAT TTG ATA TTT AAT GAA AGC CTT GGC CAA GGC ACT TTT ACA AAG
Arg Asn Glu Asp Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys

       1683        1692        1701        1710        1719        1728
ATT TTT AAA GGC GTA CGA AGA GAA GTA GGA GAC TAC GGT CAA CTG CAT GAA ACA
Ile Phe Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr

       1737        1746        1755        1764        1773        1782
GAA GTT CTT TTA AAA GTT CTG GAT AAA GCA CAC AGG AAC TAT TCA GAG TCT TTC
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu Ser Phe

       1791        1800        1809        1818        1827        1836
TTT GAA GCA GCA AGT ATG ATG AGC AAG CTT TCT CAC AAG CAT TTG GTT TTA AAT
Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His Leu Val Leu Asn

       1845        1854        1863        1872        1881        1890
TAT GGA GTA TGT GTC TGT GGA GAC GAG AAT ATT CTG GTT CAG GAG TTT GTA AAA
Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe Val Lys
```

FIGURE 1D

```
      1899        1908        1917        1926        1935        1944
TTT GGA TCA CTA GAT ACA TAT CTG AAA AAG AAT AAA AAT TGT ATA AAT ATA TTA
Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys Asn Lys Asn Cys Ile Asn Ile Leu

    1953        1962        1971        1980        1989        1998
TGG AAA CTT GAA GTT GCT AAA CAG TTG GCA TGG GCC ATG CAT TTT CTA GAA GAA
Trp Lys Leu Glu Val Ala Lys Gln Leu Ala Trp Ala Met His Phe Leu Glu Glu

      2007        2016        2025        2034        2043        2052
AAC ACC CTT ATT CAT GGG AAT GTA TGT GCC AAA AAT ATT CTG CTT ATC AGA GAA
Asn Thr Leu Ile His Gly Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu

      2061        2070        2079        2088        2097        2106
GAA GAC AGG AAG ACA GGA AAT CCT CCT TTC ATC AAA CTT AGT GAT CCT GGC ATT
Glu Asp Arg Lys Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile

      2115        2124        2133        2142        2151        2160
AGT ATT ACA GTT TTG CCA AAG GAC ATT CTT CAG GAG AGA ATA CCA TGG GTA CCA
Ser Ile Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro

      2169        2178        2187        2196        2205        2214
CCT GAA TGC ATT GAA AAT CCT AAA AAT TTA AAT TTG GCA ACA GAC AAA TGG AGT
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys Trp Ser

      2223        2232        2241        2250        2259        2268
TTT GGT ACC ACT TTG TGG GAA ATC TGC AGT GGA GGA GAT AAA CCT CTA AGT GCT
Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys Pro Leu Ser Ala

      2277        2286        2295        2304        2313        2322
CTG GAT TCT CAA AGA AAG CTA CAA TTT TAT GAA GAT AGG CAT CAG CTT CCT GCA
Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp Arg His Gln Leu Pro Ala

      2331        2340        2349        2358        2367        2376
CCA AAG TGG GCA GAA TTA GCA AAC CTT ATA AAT AAT TGT ATG GAT TAT GAA CCA
Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile Asn Asn Cys Met Asp Tyr Glu Pro
```

FIGURE 1E

```
      2385        2394        2403        2412        2421        2430
GAT TTC AGG CCT TCT TTC AGA GCC ATC ATA CGA GAT CTT AAC AGT TTG TTT ACT
Asp Phe Arg Pro Ser Phe Arg Ala Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr

      2439        2448        2457        2466        2475        2484
CCA GAT TAT GAA CTA TTA ACA GAA AAT GAC ATG TTA CCA AAT ATG AGG ATA GGT
Pro Asp Tyr Glu Leu Leu Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly

      2493        2502        2511        2520        2529        2538
GCC TTG GGG TTT TCT GGT GCC TTT GAA GAC CGG GAT CCT ACA CAG TTT GAA GAG
Ala Leu Gly Phe Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu

      2547        2556        2565        2574        2583        2592
AGA CAT TTG AAA TTT CTA CAG CAA CTT GGC AAG GGT AAT TTT GGG AGT GTG GAG
Arg His Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu

      2601        2610        2619        2628        2637        2646
ATG TGC CGG TAT GAC CCT CTA CAG GAC AAC ACT GGG GAG GTG GTC GCT GTA AAA
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala Val Lys

      2655        2664        2673        2682        2691        2700
AAG CTT CAG CAT AGT ACT GAA GAG CAC CTA AGA GAC TTT GAA AGG GAA ATT GAA
Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu Arg Glu Ile Glu

      2709        2718        2727        2736        2745        2754
ATC CTG AAA TCC CTA CAG CAT GAC AAC ATT GTA AAG TAC AAG GGA GTG TGC TAC
Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys Tyr Lys Gly Val Cys Tyr

      2763        2772        2781        2790        2799        2808
AGT GCT GGT CGG CGT AAT CTA AAA TTA ATT ATG GAA TAT TTA CCA TAT GGA AGT
Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile Met Glu Tyr Leu Pro Tyr Gly Ser

      2817        2826        2835        2844        2853        2862
TTA CGA GAC TAT CTT CAA AAA CAT AAA GAA CGG ATA GAT CAC ATA AAA CTT CTG
Leu Arg Asp Tyr Leu Gln Lys His Lys Glu Arg Ile Asp His Ile Lys Leu Leu
```

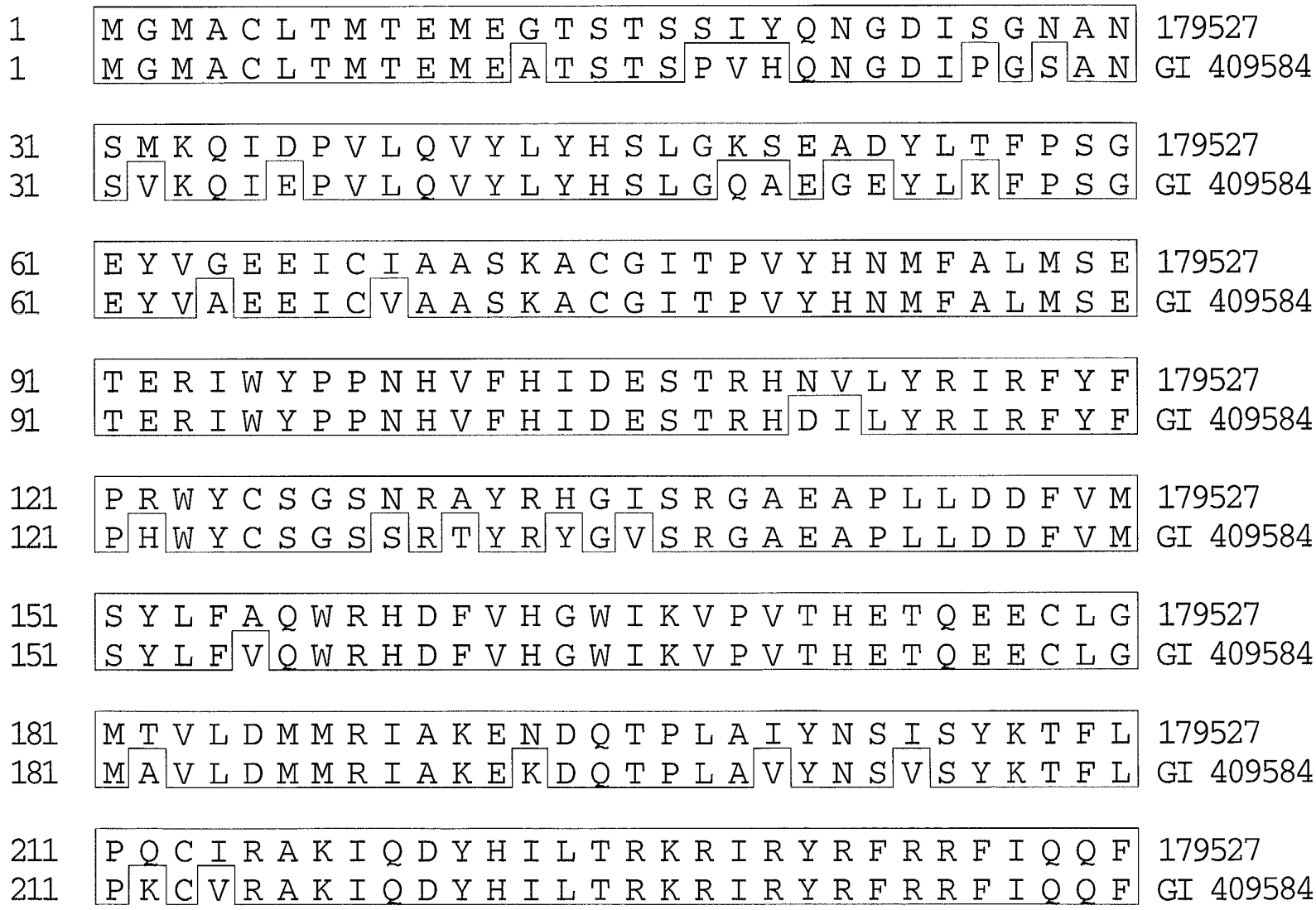
FIGURE 1F

```
        2871        2880        2889        2898        2907        2916
CAG TAC ACA TCT CAG ATA TGC AAG GGT ATG GAG TAT CTT GGT ACA AAA AGG TAT
Gln Tyr Thr Ser Gln Ile Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr

        2925        2934        2943        2952        2961        2970
ATC CAC AGG GAT CTG GCA ACG AGA AAT ATA TTG GTG GAG AAC GAG AAC AGA GTT
Ile His Arg Asp Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val

        2979        2988        2997        3006        3015        3024
AAA ATT GGR GAT TTT GGG TTA ACC AAA GTC TTG CCA CAA GAC AAA GAA TAC TAT
Lys Ile Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr

        3033        3042        3051        3060        3069        3078
AAA GTA AAA GAA CCT GGT GAA AGT CCC ATA TTC TGG TAT GCT CCA GAA TCA CTG
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Glu Ser Leu

        3087        3096        3105        3114        3123        3132
ACA GAG AGC AAG TTT TCT GTG GCC TCA GAT GTT TGG AGC TTT GGA GTG GTT CTG
Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe Gly Val Val Leu

        3141        3150        3159        3168        3177        3186
TAT GAA CTT TTC ACA TAC ATT GAG AAG AGT AAA AGT CCA CCA GCG GAA TTT ATG
Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser Pro Pro Ala Glu Phe Met

        3195        3204        3213        3222        3231        3240
CGT ATG ATT GGC AAT GAC AAA CAA GGA CAG ATG ATC GTG TTC CAT TTG ATA GAA
Arg Met Ile Gly Asn Asp Lys Gln Gly Gln Met Ile Val Phe His Leu Ile Glu

        3249        3258        3267        3276        3285        3294
CTT TTG AAG AAT AAT GGA AGA TTA CCA AGA CCA GAT GGA TGC CCA GAT GAG ATC
Leu Leu Lys Asn Asn Gly Arg Leu Pro Arg Pro Asp Gly Cys Pro Asp Glu Ile

        3303        3312        3321        3330        3339        3348
TAT ATG ATC ATG ACA GAA TGC TGG AAC AAT AAT GTA AAT CAA CGC CCC TCC TTT
Tyr Met Ile Met Thr Glu Cys Trp Asn Asn Asn Val Asn Gln Arg Pro Ser Phe
```

FIGURE 1G

```
       3357        3366        3375        3384        3393
AGG GAT CTA GCT CTT CGA GTG GAT CAA ATA AGG GAT AAC ATG GCT GGA TGA  3'
Arg Asp Leu Ala Leu Arg Val Asp Gln Ile Arg Asp Asn Met Ala Gly ***
```

FIGURE 1H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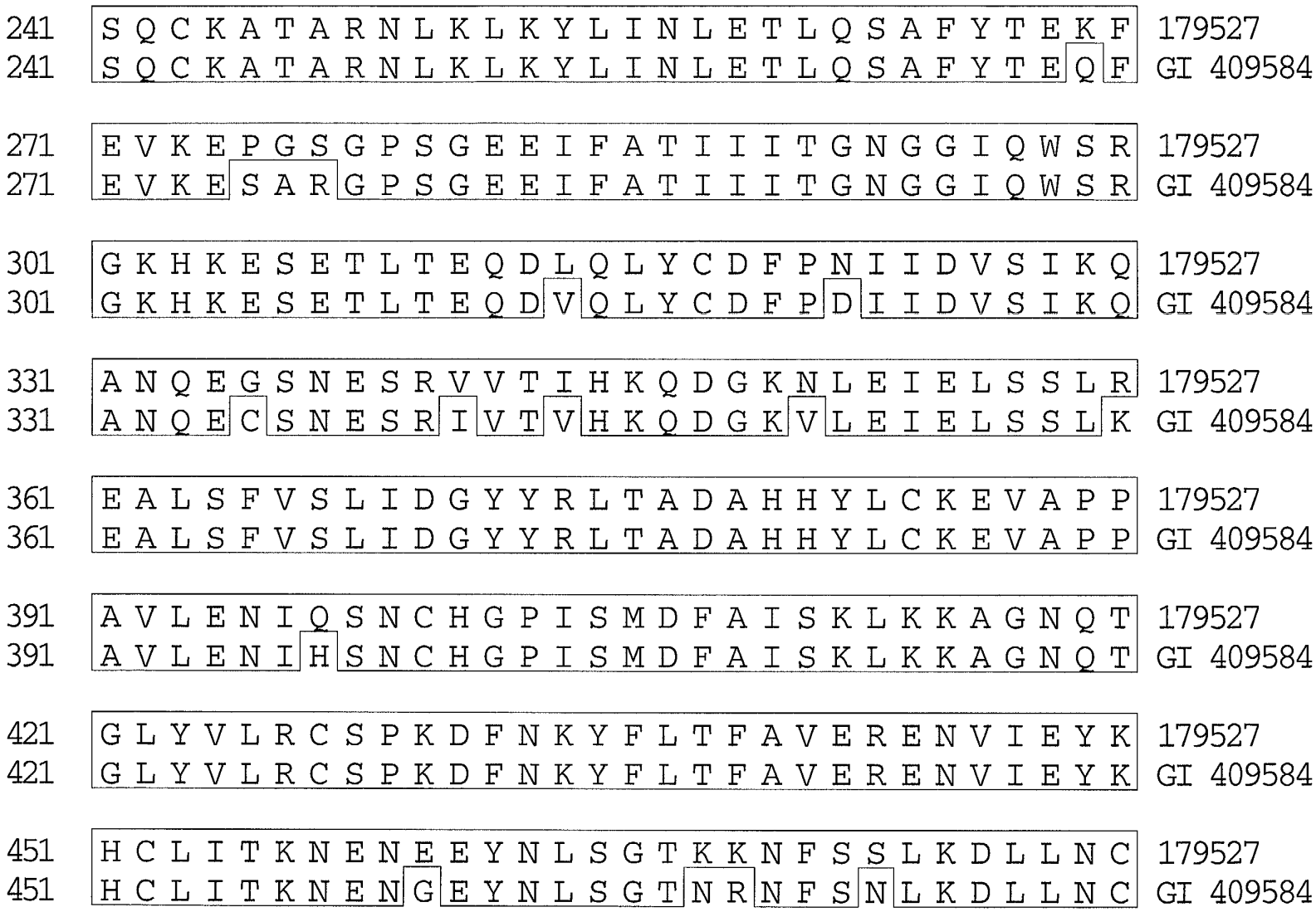
61 EYVGEEICIAASKACGITPVYHNMFALMSE 179527
61 EYVAEEICVAASKACGITPVYHNMFALMSE GI 409584
91 TERIWYPPNHVFHIDESTRHNVLYRIRFYF 179527
91 TERIWYPPNHVFHIDESTRHDILYRIRFYF GI 409584
121 PRWYCSGSNRAYRHGISRGAEAPLLDDFVM 179527
121 PHWYCSGSSRTYRYGVSRGAEAPLLDDFVM GI 409584
151 SYLFAQWRHDFVHGWIKVPVTHETQEECLG 179527
151 SYLFVQWRHDFVHGWIKVPVTHETQEECLG GI 409584
181 MTVLDMMRIAKENDQTPLAIYNSISYKTFL 179527
181 MAVLDMMRIAKEKDQTPLAVYNSVSYKTFL GI 409584
211 PQCIRAKIQDYHILTRKRIRYRFRRFIQQF 179527
211 PKCVRAKIQDYHILTRKRIRYRFRRFIQQF GI 409584

FIGURE 2A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241 SQCKATARNLKLKYLINLETLQSAFYTEQF GI 409584
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271 EVKESARGPSGEEIFATIIITGNGGIQWSR GI 409584
301 GKHKESETLTEQDLQLYCDFPNIIDVSIKQ 179527
301 GKHKESETLTEQDVQLYCDFPDIIDVSIKQ GI 409584
331 ANQEGSNESRVVTIHKQDGKNLEIELSSLR 179527
331 ANQECSNESRIVTVHKQDGKVLEIELSSLK GI 409584
361 EALSFVSLIDGYYRLTADAHHYLCKEVAPP 179527
361 EALSFVSLIDGYYRLTADAHHYLCKEVAPP GI 409584
391 AVLENIQSNCHGPISMDFAISKLKKAGNQT 179527
391 AVLENIHSNCHGPISMDFAISKLKKAGNQT GI 409584
421 GLYVLRCSPKDFNKYFLTFAVERENVIEYK 179527
421 GLYVLRCSPKDFNKYFLTFAVERENVIEYK GI 409584
451 HCLITKNENEEYNLSGTKKNFSSLKDLLNC 179527
451 HCLITKNENGEYNLSGTNRNFSNLKDLLNC GI 409584

FIGURE 2B

```
481  YQMETVRSDNIIFQFTKCCPPKPKDKSNLL  179527
481  YQMETVRSDSIIFQFTKCCPPKPKDKSNLL  GI 409584

511  VFRTNGVSDVPTSPTLQRPTHMNQMVFHKI  179527
511  VFRTNGISDVQISPTLQRHNNVNQMVFHKI  GI 409584

541  RNEDLIFNESLGQGTFTKIFKGVRREVGDY  179527
541  RNEDLIFNESLGQGTFTKIFKGVRREVGDY  GI 409584

571  GQLHETEVLLKVLDKAHRNYSESFFEAASM  179527
571  GQLHKTEVLLKVLDKAHRNYSESFFEAASM  GI 409584

601  MSKLSHKHLVLNYGVCVCGDENILVQEFVK  179527
601  MSQLSHKHLVLNYGVCVCGEENILVQEFVK  GI 409584

631  FGSLDTYLKKNKNCINILWKLEVAKQLAWA  179527
631  FGSLDTYLKKNKNSINILWKLGVAKQLAWA  GI 409584

661  MHFLEENTLIHGNVCAKNILLIREEDRKTG  179527
661  MHFLEEKSLIHGNVCAKNILLIREEDRRTG  GI 409584

691  NPPFIKLSDPGISITVLPKDILQERIPWVP  179527
691  NPPFIKLSDPGISITVLPKDILQERIPWVP  GI 409584
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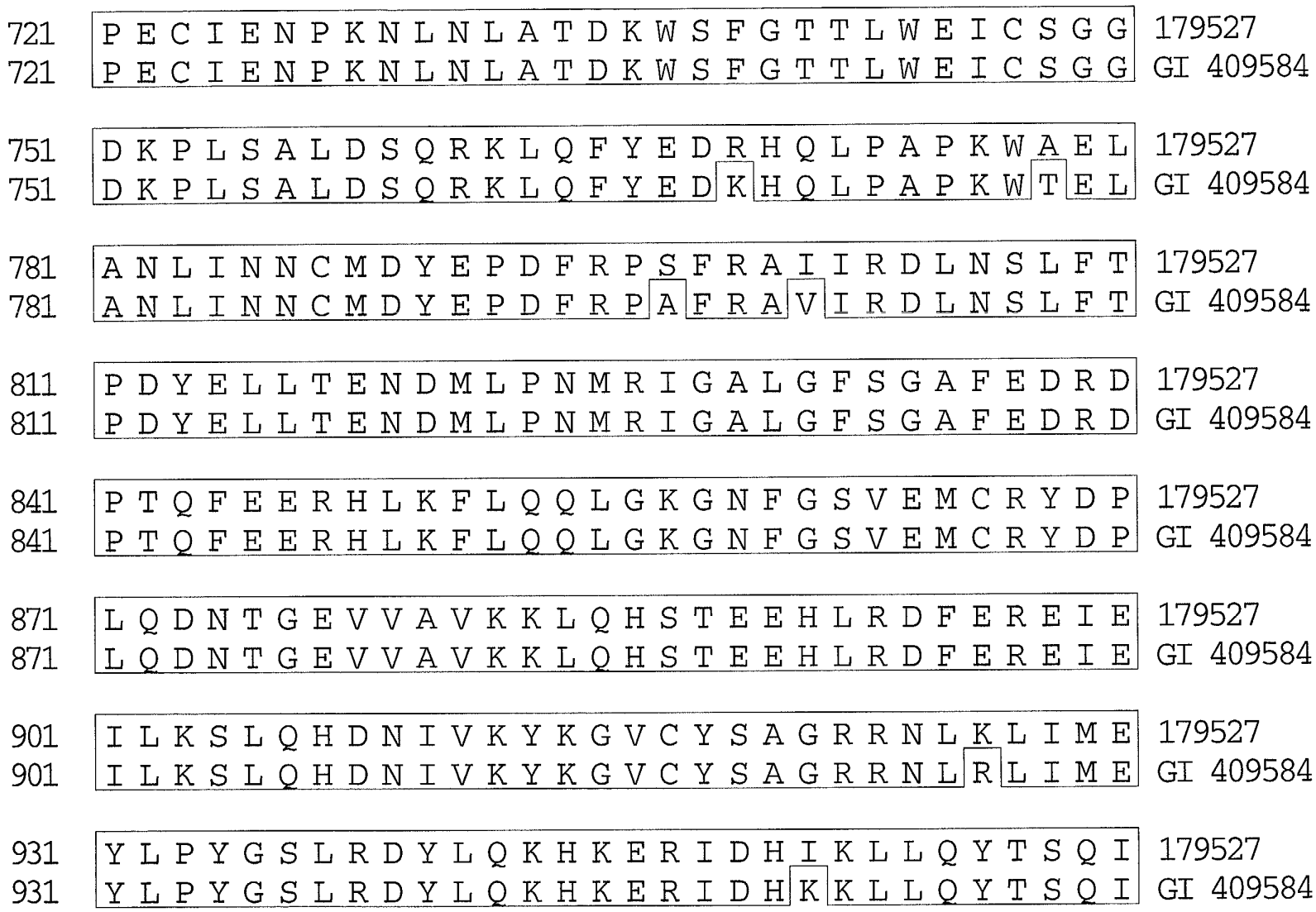
FIGURE 2C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751 D K P L S A L D S Q R K L Q F Y E D R H Q L P A P K W A E L 179527
751 D K P L S A L D S Q R K L Q F Y E D K H Q L P A P K W T E L GI 409584
781 A N L I N N C M D Y E P D F R P S F R A I I R D L N S L F T 179527
781 A N L I N N C M D Y E P D F R P A F R A V I R D L N S L F T GI 409584
811 P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D 179527
811 P D Y E L L T E N D M L P N M R I G A L G F S G A F E D R D GI 409584
841 P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P 179527
841 P T Q F E E R H L K F L Q Q L G K G N F G S V E M C R Y D P GI 409584
871 L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E 179527
871 L Q D N T G E V V A V K K L Q H S T E E H L R D F E R E I E GI 409584
901 I L K S L Q H D N I V K Y K G V C Y S A G R R N L K L I M E 179527
901 I L K S L Q H D N I V K Y K G V C Y S A G R R N L R L I M E GI 409584
931 Y L P Y G S L R D Y L Q K H K E R I D H I K L L Q Y T S Q I 179527
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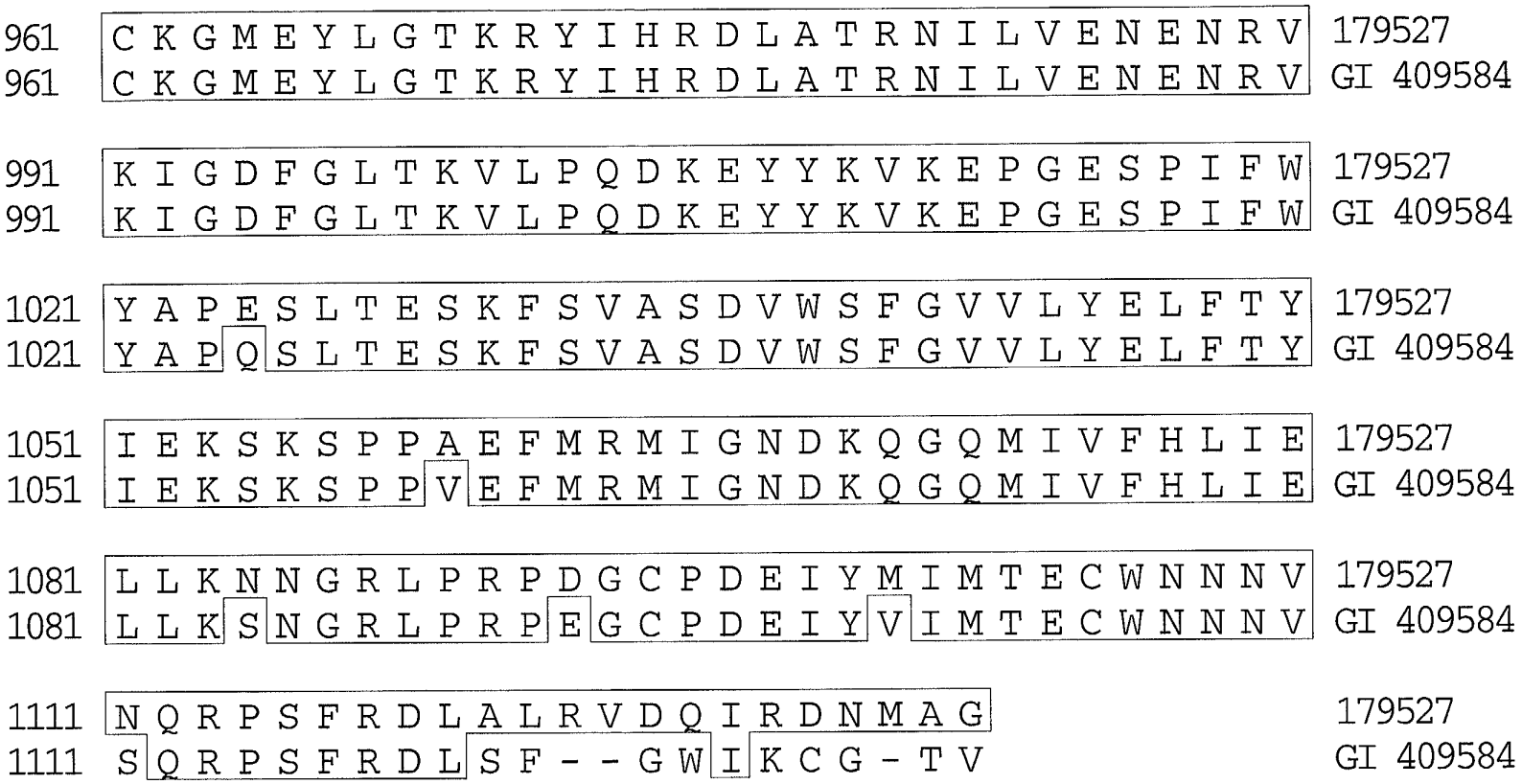
FIGURE 2D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961 C K G M E Y L G T K R Y I H R D L A T R N I L V E N E N R V GI 409584
991 K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W 179527
991 K I G D F G L T K V L P Q D K E Y Y K V K E P G E S P I F W GI 409584
1021 Y A P E S L T E S K F S V A S D V W S F G V V L Y E L F T Y 179527
1021 Y A P Q S L T E S K F S V A S D V W S F G V V L Y E L F T Y GI 409584
1051 I E K S K S P P A E F M R M I G N D K Q G Q M I V F H L I E 179527
1051 I E K S K S P P V E F M R M I G N D K Q G Q M I V F H L I E GI 409584
1081 L L K N N G R L P R P D G C P D E I Y M I M T E C W N N N V 179527
1081 L L K S N G R L P R P E G C P D E I Y V I M T E C W N N N V GI 409584
1111 N Q R P S F R D L A L R V D Q I R D N M A G 179527
1111 S Q R P S F R D L S F - - G W I K C G - T V GI 409584

FIGURE 2E

```
          348         357         366         375         384         393
5' ATG GGA ATG GCC TGC CTT ACG ATG ACA GAA ATG GAG GGA ACA TCC ACC TCT TCT
   Met Gly Met Ala Cys Leu Thr Met Thr Glu Met Glu Gly Thr Ser Thr Ser Ser

          402         411         420         429         438         447
   ATA TAT CAG AAT GGT GAT ATT TCT GGA AAT GCC AAT TCT ATG AAG CAA ATA GAT
   Ile Tyr Gln Asn Gly Asp Ile Ser Gly Asn Ala Asn Ser Met Lys Gln Ile Asp

          456         465         474         483         492         501
   CCA GTT CTT CAG GTG TAT CTT TAC CAT TCC CTT GGG AAA TCT GAG GCA GAT TAT
   Pro Val Leu Gln Val Tyr Leu Tyr His Ser Leu Gly Lys Ser Glu Ala Asp Tyr

          510         519         528         537         546         555
   CTG ACC TTT CCA TCT GGG GAG TAT GTT GGA GAA GAA ATC TGT ATT GCT GCT TCT
   Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly Glu Glu Ile Cys Ile Ala Ala Ser

          564         573         582         591         600         609
   AAA GCT TGT GGT ATC ACA CCT GTG TAT CAT AAT ATG TTT GCT TTA ATG AGT GAA
   Lys Ala Cys Gly Ile Thr Pro Val Tyr His Asn Met Phe Ala Leu Met Ser Glu

          618         627         636         645         654         663
   ACA GAA AGG ATC TGG TAT CCA CCC AAC CAT GTC TTC CAT ATA GAT GAG TCA ACC
   Thr Glu Arg Ile Trp Tyr Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr

          672         681         690         699         708         717
   AGG CAT AAT GTA CTC TAC AGA ATA AGA TTT TAC TTT CCT CGT TGG TAT TGC AGT
   Arg His Asn Val Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Arg Trp Tyr Cys Ser

          726         735         744         753         762         771
   GGC AGC AAC AGA GCC TAT CGG CAT GGA ATA TCT CGA GGT GCT GAA GCT CCT CTT
   Gly Ser Asn Arg Ala Tyr Arg His Gly Ile Ser Arg Gly Ala Glu Ala Pro Leu

          780         789         798         807         816         825
   CTT GAT GAC TTT GTC ATG TCT TAC CTC TTT GCT CAG TGG CGG CAT GAT TTT GTG
   Leu Asp Asp Phe Val Met Ser Tyr Leu Phe Ala Gln Trp Arg His Asp Phe Val

          834         843         852         861         870         879
   CAT GGA TGG ATA AAA GTA CCT GTG ACT CAT GAA ACA CAG GAA GAA TGT CTT GGG
   His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu Glu Cys Leu Gly

          888         897         906         915         924         933
   ATG ACA GTG TTA GAT ATG ATG AGA ATA GCC AAA GAA AAC GAT CAA ACC CCA CTG
   Met Thr Val Leu Asp Met Met Arg Ile Ala Lys Glu Asn Asp Gln Thr Pro Leu

          942         951         960         969         978         987
   GCC ATC TAT AAC TCT ATC AGC TAC AAG ACA TTC TTA CCA CAA TGT ATT CGA GCA
   Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr Phe Leu Pro Gln Cys Ile Arg Ala
```

FIGURE 1A

```
         996        1005        1014        1023        1032        1041
AAG ATC CAA GAC TAT CAT ATT TTG ACA AGG AAG CGA ATA AGG TAC AGA TTT CGC
Lys Ile Gln Asp Tyr His Ile Leu Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg

        1050        1059        1068        1077        1086        1095
AGA TTT ATT CAG CAA TTC AGC CAA TGC AAA GCC ACT GCC AGA AAC TTG AAA CTT
Arg Phe Ile Gln Gln Phe Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu

        1104        1113        1122        1131        1140        1149
AAG TAT CTT ATA AAT CTG GAA ACT CTG CAG TCT GCC TTC TAC ACA GAG AAA TTT
Lys Tyr Leu Ile Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Lys Phe

        1158        1167        1176        1185        1194        1203
GAA GTA AAA GAA CCT GGA AGT GGT CCT TCA GGT GAG GAG ATT TTT GCA ACC ATT
Glu Val Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile

        1212        1221        1230        1239        1248        1257
ATA ATA ACT GGA AAC GGT GGA ATT CAG TGG TCA AGA GGG AAA CAT AAA GAA AGT
Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys Glu Ser

        1266        1275        1284        1293        1302        1311
GAG ACA CTG ACA GAA CAG GAT TTA CAG TTA TAT TGC GAT TTT CCT AAT ATT ATT
Glu Thr Leu Thr Glu Gln Asp Leu Gln Leu Tyr Cys Asp Phe Pro Asn Ile Ile

        1320        1329        1338        1347        1356        1365
GAT GTC AGT ATT AAG CAA GCA AAC CAA GAG GGT TCA AAT GAA AGC CGA GTT GTA
Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Gly Ser Asn Glu Ser Arg Val Val

        1374        1383        1392        1401        1410        1419
ACT ATC CAT AAG CAA GAT GGT AAA AAT CTG GAA ATT GAA CTT AGC TCA TTA AGG
Thr Ile His Lys Gln Asp Gly Lys Asn Leu Glu Ile Glu Leu Ser Ser Leu Arg

        1428        1437        1446        1455        1464        1473
GAA GCT TTG TCT TTC GTG TCA TTA ATT GAT GGA TAT TAT AGA TTA ACT GCA GAT
Glu Ala Leu Ser Phe Val Ser Leu Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp

        1482        1491        1500        1509        1518        1527
GCA CAT CAT TAC CTC TGT AAA GAA GTA GCA CCT CCA GCC GTG CTT GAA AAT ATA
Ala His His Tyr Leu Cys Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile

        1536        1545        1554        1563        1572        1581
CAA AGC AAC TGT CAT GGC CCA ATT TCG ATG GAT TTT GCC ATT AGT AAA CTG AAG
Gln Ser Asn Cys His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys

        1590        1599        1608        1617        1626        1635
AAA GCA GGT AAT CAG ACT GGA CTG TAT GTA CTT CGA TGC AGT CCT AAG GAC TTT
Lys Ala Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
```

FIGURE 1B

```
        1644        1653        1662        1671        1680        1689
AAT AAA TAT TTT TTG ACT TTT GCT GTC GAG CGA GAA AAT GTC ATT GAA TAT AAA
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu Tyr Lys

        1698        1707        1716        1725        1734        1743
CAC TGT TTG ATT ACA AAA AAT GAG AAT GAA GAG TAC AAC CTC AGT GGG ACA AAG
His Cys Leu Ile Thr Lys Asn Glu Asn Glu Glu Tyr Asn Leu Ser Gly Thr Lys

        1752        1761        1770        1779        1788        1797
AAG AAC TTC AGC AGT CTT AAA GAT CTT TTG AAT TGT TAC CAG ATG GAA ACT GTT
Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys Tyr Gln Met Glu Thr Val

        1806        1815        1824        1833        1842        1851
CGC TCA GAC AAT ATA ATT TTC CAG TTT ACT AAA TGC TGT CCC CCA AAG CCA AAA
Arg Ser Asp Asn Ile Ile Phe Gln Phe Thr Lys Cys Cys Pro Pro Lys Pro Lys

        1860        1869        1878        1887        1896        1905
GAT AAA TCA AAC CTT CTA GTC TTC AGA ACG AAT GGT GTT TCT GAT GTA CCA ACC
Asp Lys Ser Asn Leu Leu Val Phe Arg Thr Asn Gly Val Ser Asp Val Pro Thr

        1914        1923        1932        1941        1950        1959
TCA CCA ACA TTA CAG AGG CCT ACT CAT ATG AAC CAA ATG GTG TTT CAC AAA ATC
Ser Pro Thr Leu Gln Arg Pro Thr His Met Asn Gln Met Val Phe His Lys Ile

        1968        1977        1986        1995        2004        2013
AGA AAT GAA GAT TTG ATA TTT AAT GAA AGC CTT GGC CAA GGC ACT TTT ACA AAG
Arg Asn Glu Asp Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys

        2022        2031        2040        2049        2058        2067
ATT TTT AAA GGC GTA CGA AGA GAA GTA GGA GAC TAC GGT CAA CTG CAT GAA ACA

Ile Phe Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr

        2076        2085        2094        2103        2112        2121
GAA GTT CTT TTA AAA GTT CTG GAT AAA GCA CAC AGG AAC TAT TCA GAG TCT TTC
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu Ser Phe

        2130        2139        2148        2157        2166        2175
TTT GAA GCA GCA AGT ATG ATG AGC AAG CTT TCT CAC AAG CAT TTG GTT TTA AAT
Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His Leu Val Leu Asn

        2184        2193        2202        2211        2220        2229
TAT GGA GTA TGT GTC TGT GGA GAC GAG AAT ATT CTG GTT CAG GAG TTT GTA AAA
Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe Val Lys

        2238        2247        2256        2265        2274        2283
TTT GGA TCA CTA GAT ACA TAT CTG AAA AAG AAT AAA AAT TGT ATA AAT ATA TTA
Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys Asn Lys Asn Cys Ile Asn Ile Leu
```

FIGURE 1C

```
        2292        2301        2310        2319        2328        2337
TGG AAA CTT GAA GTT GCT AAA CAG TTG GCA TGG GCC ATG CAT TTT CTA GAA GAA
Trp Lys Leu Glu Val Ala Lys Gln Leu Ala Trp Ala Met His Phe Leu Glu Glu

        2346        2355        2364        2373        2382        2391
AAC ACC CTT ATT CAT GGG AAT GTA TGT GCC AAA AAT ATT CTG CTT ATC AGA GAA
Asn Thr Leu Ile His Gly Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu

        2400        2409        2418        2427        2436        2445
GAA GAC AGG AAG ACA GGA AAT CCT CCT TTC ATC AAA CTT AGT GAT CCT GGC ATT
Glu Asp Arg Lys Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile

        2454        2463        2472        2481        2490        2499
AGT ATT ACA GTT TTG CCA AAG GAC ATT CTT CAG GAG AGA ATA CCA TGG GTA CCA
Ser Ile Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro

        2508        2517        2526        2535        2544        2553
CCT GAA TGC ATT GAA AAT CCT AAA AAT TTA AAT TTG GCA ACA GAC AAA TGG AGT
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys Trp Ser

        2562        2571        2580        2589        2598        2607
TTT GGT ACC ACT TTG TGG GAA ATC TGC AGT GGA GGA GAT AAA CCT CTA AGT GCT
Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys Pro Leu Ser Ala

        2616        2625        2634        2643        2652        2661
CTG GAT TCT CAA AGA AAG CTA CAA TTT TAT GAA GAT AGG CAT CAG CTT CCT GCA
Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp Arg His Gln Leu Pro Ala

        2670        2679        2688        2697        2706        2715
CCA AAG TGG GCA GAA TTA GCA AAC CTT ATA AAT AAT TGT ATG GAT TAT GAA CCA
Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile Asn Asn Cys Met Asp Tyr Glu Pro

        2724        2733        2742        2751        2760        2769
GAT TTC AGG CCT TCT TTC AGA GCC ATC ATA CGA GAT CTT AAC AGT TTG TTT ACT
Asp Phe Arg Pro Ser Phe Arg Ala Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr

        2778        2787        2796        2805        2814        2823
CCA GAT TAT GAA CTA TTA ACA GAA AAT GAC ATG TTA CCA AAT ATG AGG ATA GGT
Pro Asp Tyr Glu Leu Leu Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly

        2832        2841        2850        2859        2868        2877
GCC TTG GGG TTT TCT GGT GCC TTT GAA GAC CGG GAT CCT ACA CAG TTT GAA GAG
Ala Leu Gly Phe Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu

        2886        2895        2904        2913        2922        2931
```

FIGURE 1D

```
AGA CAT TTG AAA TTT CTA CAG CAA CTT GGC AAG GGT AAT TTT GGG AGT GTG GAG
Arg His Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu

       2940        2949        2958        2967        2976        2985
ATG TGC CGG TAT GAC CCT CTA CAG GAC AAC ACT GGG GAG GTG GTC GCT GTA AAA
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala Val Lys

       2994        3003        3012        3021        3030        3039
AAG CTT CAG CAT AGT ACT GAA GAG CAC CTA AGA GAC TTT GAA AGG GAA ATT GAA
Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu Arg Glu Ile Glu

       3048        3057        3066        3075        3084        3093
ATC CTG AAA TCC CTA CAG CAT GAC AAC ATT GTA AAG TAC AAG GGA GTG TGC TAC
Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys Tyr Lys Gly Val Cys Tyr

       3102        3111        3120        3129        3138        3147
AGT GCT GGT CGG CGT AAT CTA AAA TTA ATT ATG GAA TAT TTA CCA TAT GGA AGT
Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile Met Glu Tyr Leu Pro Tyr Gly Ser

       3156        3165        3174        3183        3192        3201
TTA CGA GAC TAT CTT CAA AAA CAT AAA GAA CGG ATA GAT CAC ATA AAA CTT CTG
Leu Arg Asp Tyr Leu Gln Lys His Lys Glu Arg Ile Asp His Ile Lys Leu Leu

       3210        3219        3228        3237        3246        3255
CAG TAC ACA TCT CAG ATA TGC AAG GGT ATG GAG TAT CTT GGT ACA AAA AGG TAT
Gln Tyr Thr Ser Gln Ile Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr

       3264        3273        3282        3291        3300        3309
ATC CAC AGG GAT CTG GCA ACG AGA AAT ATA TTG GTG GAG AAC GAG AAC AGA GTT
Ile His Arg Asp Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val

       3318        3327        3336        3345        3354        3363
AAA ATT GGA GAT TTT GGG TTA ACC AAA GTC TTG CCA CAA GAC AAA GAA TAC TAT
Lys Ile Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr

       3372        3381        3390        3399        3408        3417
AAA GTA AAA GAA CCT GGT GAA AGT CCC ATA TTC TGG TAT GCT CCA GAA TCA CTG
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Glu Ser Leu

       3426        3435        3444        3453        3462        3471
ACA GAG AGC AAG TTT TCT GTG GCC TCA GAT GTT TGG AGC TTT GGA GTG GTT CTG
Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe Gly Val Val Leu

       3480        3489        3498        3507        3516        3525
TAT GAA CTT TTC ACA TAC ATT GAG AAG AGT AAA AGT CCA CCA GCG GAA TTT ATG
Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser Pro Pro Ala Glu Phe Met

       3534        3543        3552        3561        3570        3579
CGT ATG ATT GGC AAT GAC AAA CAA GGA CAG ATG ATC GTG TTC CAT TTG ATA GAA
Arg Met Ile Gly Asn Asp Lys Gln Gly Gln Met Ile Val Phe His Leu Ile Glu
```

FIGURE 1E

```
        3588        3597        3606        3615        3624        3633
CTT TTG AAG AAT AAT GGA AGA TTA CCA AGA CCA GAT GGA TGC CCA GAT GAG ATC
Leu Leu Lys Asn Asn Gly Arg Leu Pro Arg Pro Asp Gly Cys Pro Asp Glu Ile

        3642        3651        3660        3669        3678        3687
TAT ATG ATC ATG ACA GAA TGC TGG AAC AAT AAT GTA AAT CAA CGC CCC TCC TTT
Tyr Met Ile Met Thr Glu Cys Trp Asn Asn Asn Val Asn Gln Arg Pro Ser Phe

        3696        3705        3714        3723        3732
AGG GAT CTA GCT CTT CGA GTG GAT CAA ATA AGG GAT AAC ATG GCT GGA TGA  3'
Arg Asp Leu Ala Leu Arg Val Asp Gln Ile Arg Asp Asn Met Ala Gly ***
```

FIGURE 1F

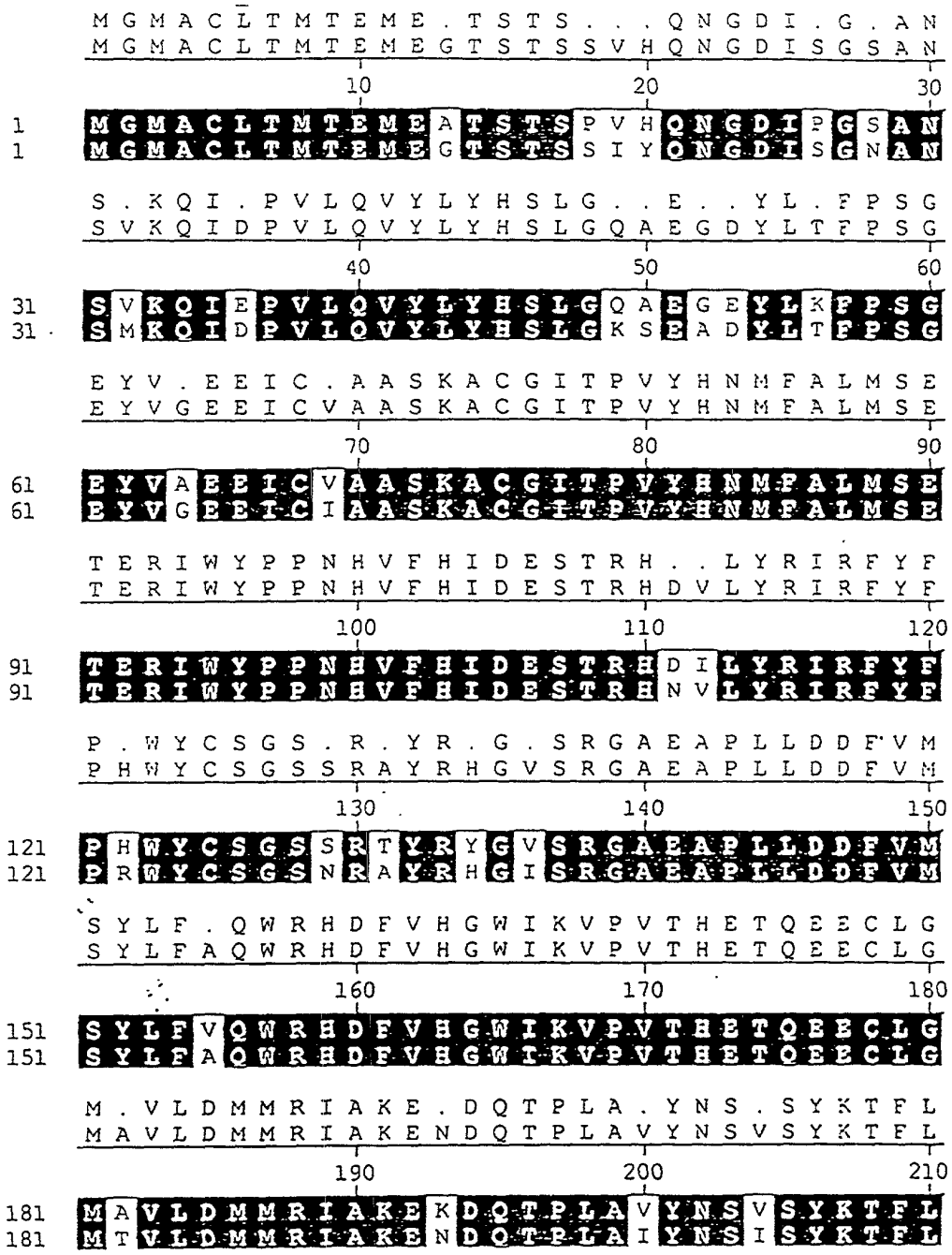
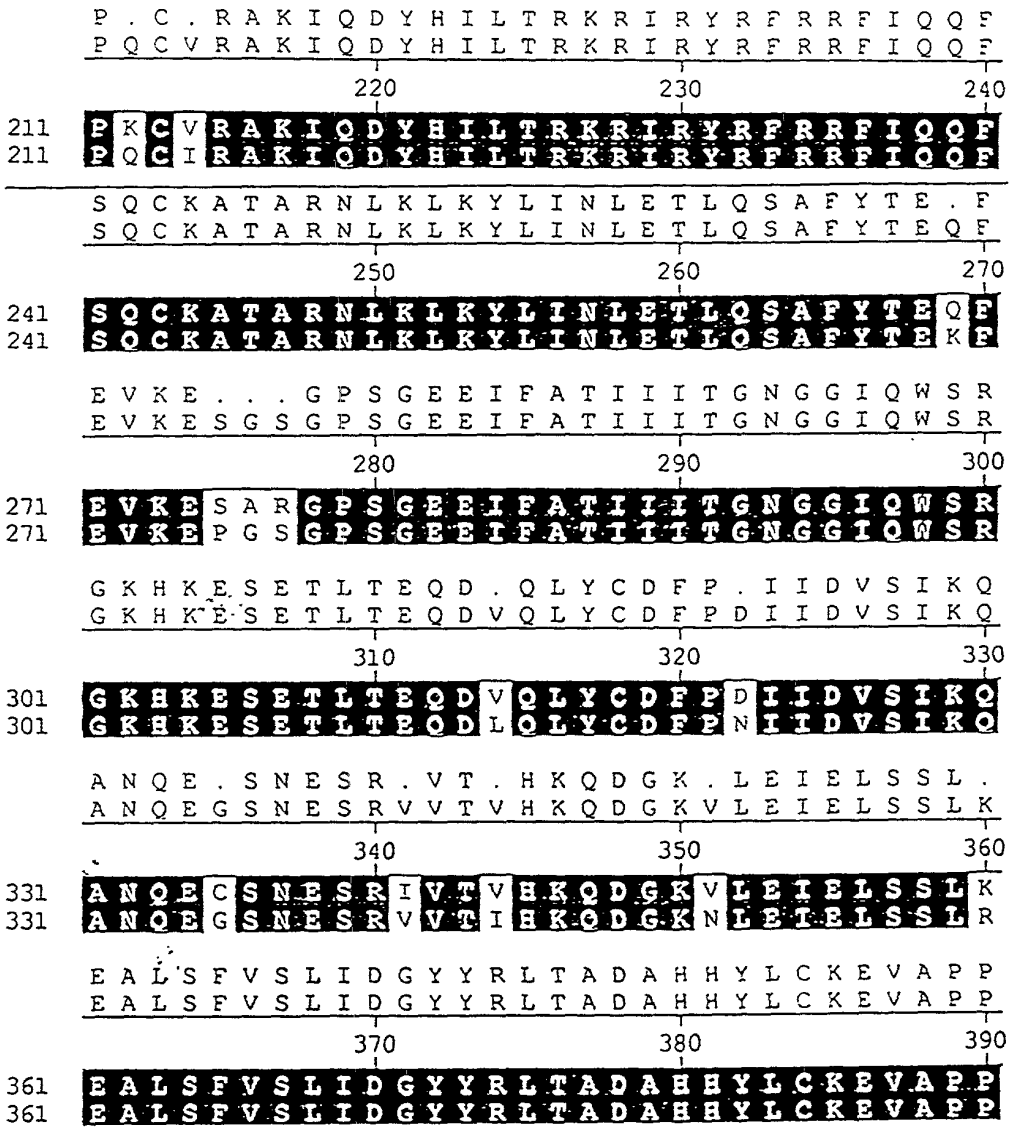


FIGURE 2A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241 S Q C K A T A R N L K L K Y L I N L E T L Q S A F Y T E Q F
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E V K E S G S G P S G E E I F A T I I I T G N G G I Q W S R
280
290
300
271 E V K E S A R G P S G E E I F A T I I I T G N G G I Q W S R
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310
320
330
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FIGURE 2B

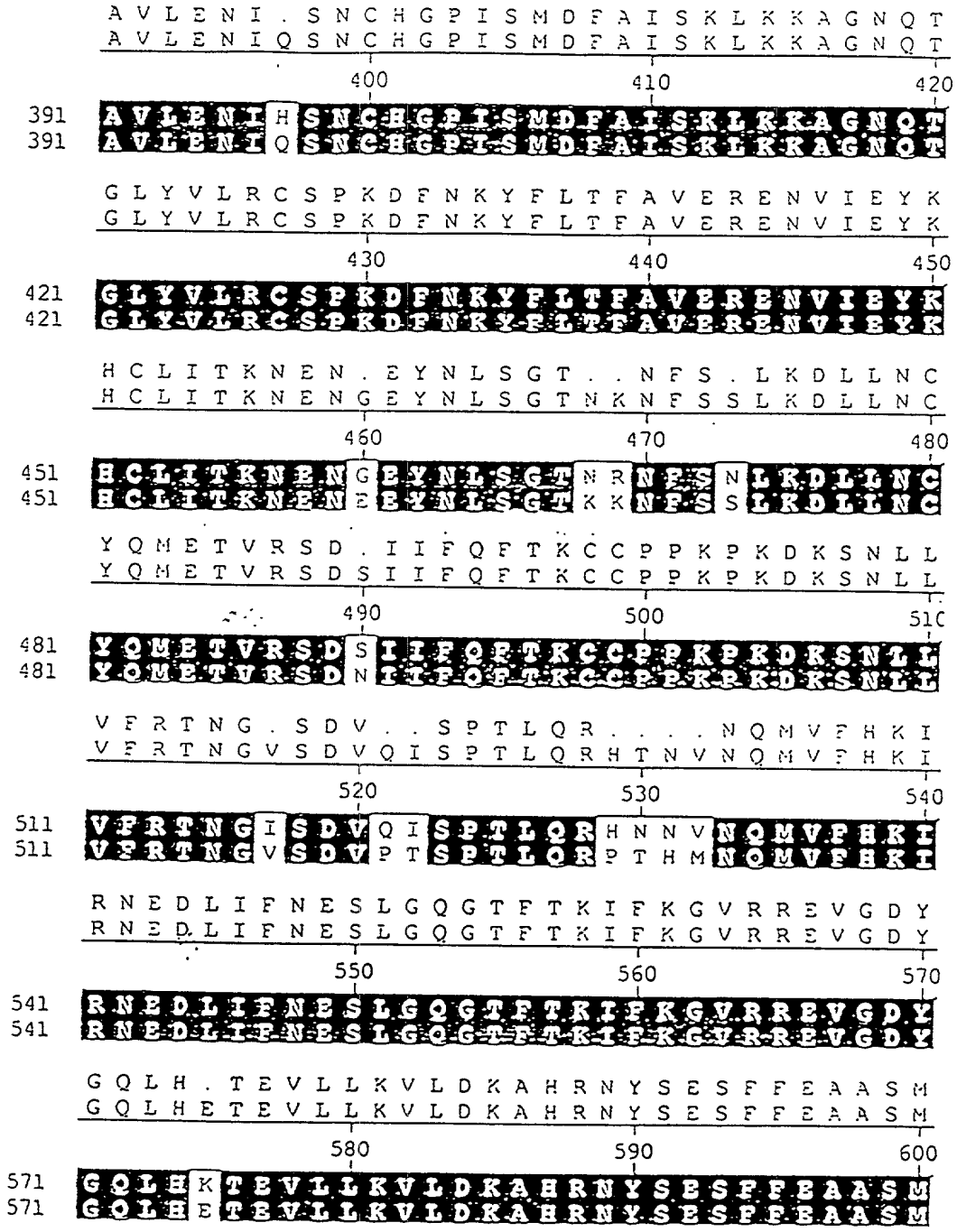
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FIGURE 2C

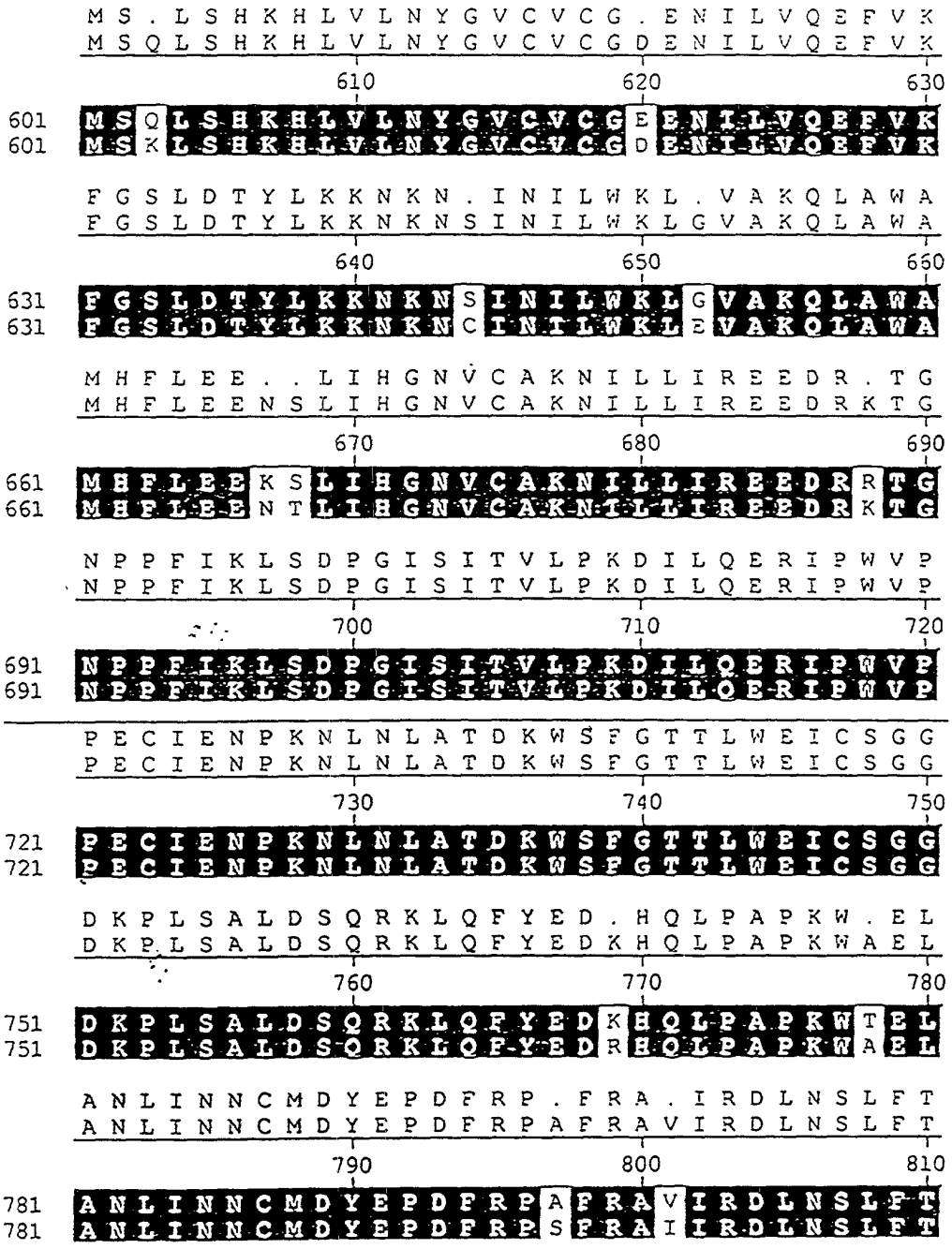

FIGURE 2D

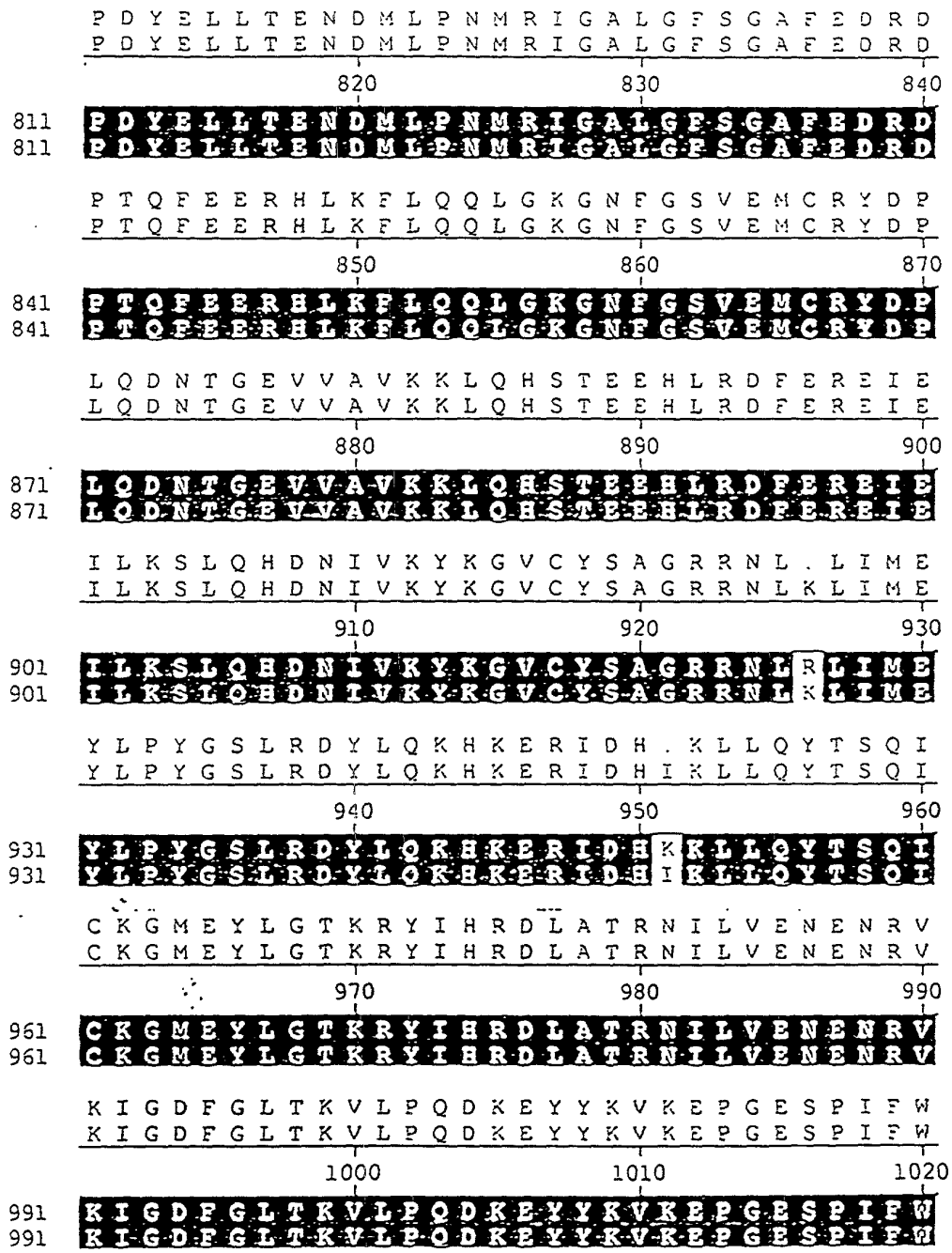
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FIGURE 2E

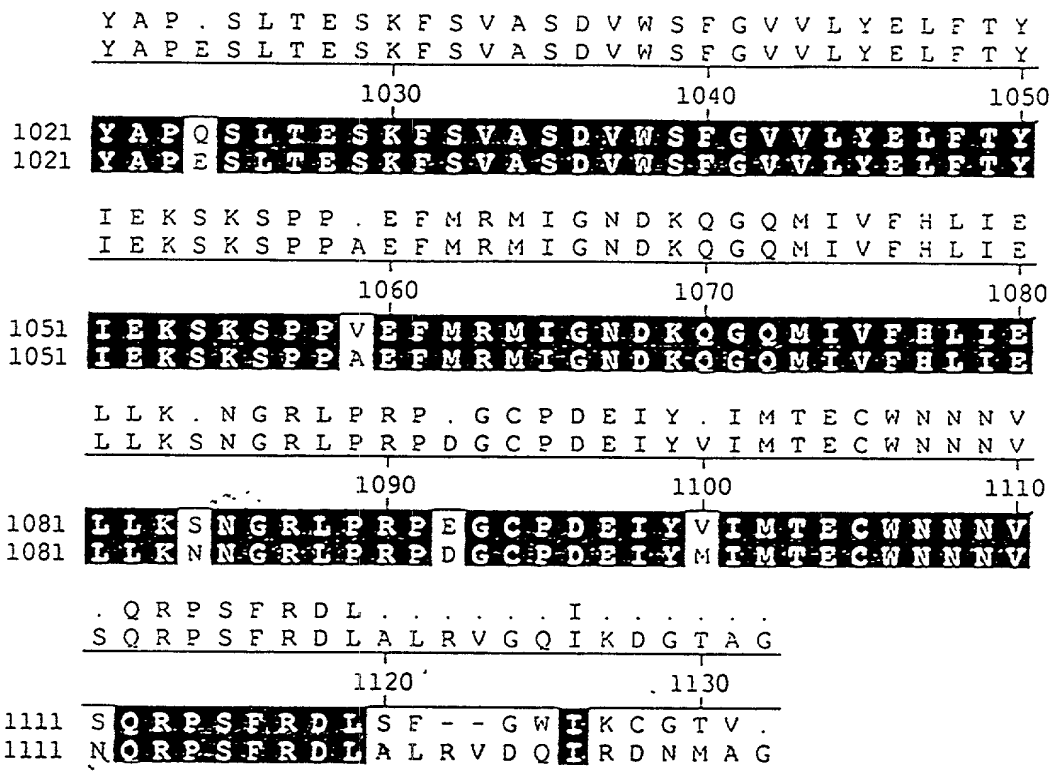
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FIGURE 2F

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

A NOVEL HUMAN JAK2 KINASE

the specification of which

/_/ is attached hereto.

/X/ was filed on December 5, 1995 as application Serial No. 08/567,508 and if this box contains an /___/, was amended on _____

/_/ was filed as Patent Cooperation Treaty international application No. _____ on _____, 19__, if this box contains an X /_/, was amended on under Patent Cooperation Treaty Article 19 on _____19_, and if this box contains an X /_/, was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international applications(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

Country	Number	Filing Date	Priority Claimed
			/_/ Yes /_/ No
			/_/ Yes /_/ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and of any Patent Cooperation Treaty international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)

I hereby appoint the following:

BARBARA J. LUTHER **Registration No.: 33,954**
DEBRA J. GLAISTER **Registration No.: 33,888**

respectively and individually, as my attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

BARBARA J. LUTHER, ESQ.
INCYTE PHARMACEUTICALS, INC.
3174 PORTER DRIVE, PALO ALTO, CALIFORNIA 94304

TEL. : 415-855-0555 FAX: 415-852-0195

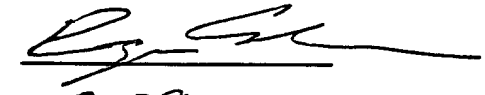
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

**IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.*

Sole Inventor or First Joint Inventor:

Full name: **ROGER COLEMAN**

Signature: 

Date: 2-29 , 1996

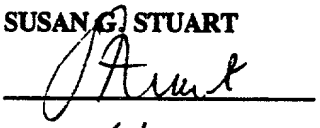
Citizenship: United States of America

Residence: Mountain View, California

P.O. Address: 260 Mariposa, #2
Mountain View, California 94041

Second Joint Inventor:

Full name: **SUSAN G. STUART**

Signature: 

Date: 27 February 1996

Citizenship: United States of America

Residence: Montara, California

P.O. Address: 1256 Birch Street
Montara, California 94037

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Box Issue Fee, Washington, D.C. 20231 on October 21, 1999.
By: _____
Printed: Nancy L. Glynn

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Coleman and Stuart

Title: NOVEL HUMAN JAK2 KINASE

Serial No.:	09/196,480	Filing Date:	November 19, 1998
Examiner:	Hutson, R.	Group Art Unit:	1652

Assistant Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

CERTIFICATE UNDER 37 C.F.R. §3.73(b), REVOCATION OF POWER OF ATTORNEY AND APPOINTMENT OF NEW ATTORNEYS

Sir:

The undersigned has reviewed all the documents in the chain of title of the above-identified patent application and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

Incyte Pharmaceuticals, Inc., having a principal place of business located at 3174 Porter Drive, Palo Alto, California 94304, certifies that it is the assignee and owner of the entire right, title and interest in, to, and under the invention described and claimed in the above-identified application by virtue of an Assignment recorded at Reel 7984, Frame 0461, hereby revokes all previous powers of attorney and appoints the following patent attorneys/agents:

Narinder S. Banait	**Reg. No. 43,482**
Adam Warwick Bell	**Reg. No. 43,490**
Lucy J. Billings	**Reg. No. 36,749**
Michael C. Cerrone	**Reg. No. 39,132**
Diana Hamlet-Cox	**Reg. No. 33,302**
Colette C. Muenzen	**Reg. No. 39,784**
Lynn E. Murry	**Reg. No. 42,918**

Docket No.: PF-0049-1 DIV

Danielle M. Pasqualone	**Reg. No. 43,847**
Susan K. Sather	**Reg. No. 44,316**
David G. Streeter	**Reg. No. 43,168**

Please direct all correspondence to:

Legal Department
Incyte Pharmaceuticals, Inc.
3174 Porter Drive
Palo Alto, California 94304

and direct all telephone calls and facsimile transmissions to: Diana Hamlet-Cox, Incyte Pharmaceuticals, Inc., Phone: (650) 855-0555, Fax: (650) 845-4166.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

INCYTE PHARMACEUTICALS, INC.

Date: October 21, 1999

By: Lee Bendekgey
Lee Bendekgey
VP, General Counsel/Corporate Secretary

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Coleman, Roger
Stuart, Susan G.

(ii) TITLE OF THE INVENTION: A NOVEL HUMAN JAK2 KINASE

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: US
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/567,508
(B) FILING DATE: 05-DEC-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Billings, Lucy J.
(B) REGISTRATION NUMBER: 36,749
(C) REFERENCE/DOCKET NUMBER: PF-0049US

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-855-0555
(B) TELEFAX: 650-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4482 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Placenta
(B) CLONE: 179527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1132 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Lys Ser Glu Ala Asp Tyr Leu Thr Phe Pro Ser Gly Glu Tyr Val Gly
    50                  55                  60
Glu Glu Ile Cys Ile Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65                  70                  75                  80
Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
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Asn Asp Gln Thr Pro Leu Ala Ile Tyr Asn Ser Ile Ser Tyr Lys Thr
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Phe Leu Pro Gln Cys Ile Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
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Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225                 230                 235                 240
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Lys Glu Pro Gly Ser Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
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Ser Gly Thr Lys Lys Asn Phe Ser Ser Leu Lys Asp Leu Leu Asn Cys
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485 490 495
Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
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Arg Thr Asn Gly Val Ser Asp Val Pro Thr Ser Pro Thr Leu Gln Arg
515 520 525
Pro Thr His Met Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
530 535 540
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545 550 555 560
Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Glu Thr
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580 585 590
Ser Phe Phe Glu Ala Ala Ser Met Met Ser Lys Leu Ser His Lys His
595 600 605
Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu
610 615 620
Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
625 630 635 640
Asn Lys Asn Cys Ile Asn Ile Leu Trp Lys Leu Glu Val Ala Lys Gln
645 650 655
Leu Ala Trp Ala Met His Phe Leu Glu Glu Asn Thr Leu Ile His Gly
660 665 670
Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Lys
675 680 685
Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
690 695 700
Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
705 710 715 720
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
725 730 735
Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
740 745 750
Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
755 760 765
Arg His Gln Leu Pro Ala Pro Lys Trp Ala Glu Leu Ala Asn Leu Ile
770 775 780
Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ser Phe Arg Ala
785 790 795 800
Ile Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
805 810 815
Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
820 825 830
Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
835 840 845
Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
850 855 860
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
865 870 875 880
Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
885 890 895
Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
900 905 910
Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Lys Leu Ile

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945                 950                 955                 960
Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
                965                 970                 975
Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
            980                 985                 990
Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr
        995                1000                1005
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Glu
   1010                1015                1020
Ser Leu Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe
025                 1030                1035                1040
Gly Val Val Leu Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser
               1045                1050                1055
Pro Pro Ala Glu Phe Met Arg Met Ile Gly Asn Asp Lys Gln Gly Gln
           1060                1065                1070
Met Ile Val Phe His Leu Ile Glu Leu Leu Lys Asn Asn Gly Arg Leu
       1075                1080                1085
Pro Arg Pro Asp Gly Cys Pro Asp Glu Ile Tyr Met Ile Met Thr Glu
   1090                1095                1100
Cys Trp Asn Asn Asn Val Asn Gln Arg Pro Ser Phe Arg Asp Leu Ala
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1129 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Gln Ala Glu Gly Glu Tyr Leu Lys Phe Pro Ser Gly Glu Tyr Val Ala
    50                  55                  60
Glu Glu Ile Cys Val Ala Ala Ser Lys Ala Cys Gly Ile Thr Pro Val
65                  70                  75                  80
Tyr His Asn Met Phe Ala Leu Met Ser Glu Thr Glu Arg Ile Trp Tyr
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Pro Pro Asn His Val Phe His Ile Asp Glu Ser Thr Arg His Asp Ile
            100                 105                 110
Leu Tyr Arg Ile Arg Phe Tyr Phe Pro His Trp Tyr Cys Ser Gly Ser
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Ser Arg Thr Tyr Arg Tyr Gly Val Ser Arg Gly Ala Glu Ala Pro Leu
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145                 150                 155                 160
Phe Val His Gly Trp Ile Lys Val Pro Val Thr His Glu Thr Gln Glu
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Phe Leu Pro Lys Cys Val Arg Ala Lys Ile Gln Asp Tyr His Ile Leu
    210                 215                 220
Thr Arg Lys Arg Ile Arg Tyr Arg Phe Arg Arg Phe Ile Gln Gln Phe
225                 230                 235                 240
Ser Gln Cys Lys Ala Thr Ala Arg Asn Leu Lys Leu Lys Tyr Leu Ile
                245                 250                 255
Asn Leu Glu Thr Leu Gln Ser Ala Phe Tyr Thr Glu Gln Phe Glu Val
            260                 265                 270
Lys Glu Ser Ala Arg Gly Pro Ser Gly Glu Glu Ile Phe Ala Thr Ile
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Ile Ile Thr Gly Asn Gly Gly Ile Gln Trp Ser Arg Gly Lys His Lys
    290                 295                 300
Glu Ser Glu Thr Leu Thr Glu Gln Asp Val Gln Leu Tyr Cys Asp Phe
305                 310                 315                 320
Pro Asp Ile Ile Asp Val Ser Ile Lys Gln Ala Asn Gln Glu Cys Ser
                325                 330                 335
Asn Glu Ser Arg Ile Val Thr Val His Lys Gln Asp Gly Lys Val Leu
            340                 345                 350
Glu Ile Glu Leu Ser Ser Leu Lys Glu Ala Leu Ser Phe Val Ser Leu
        355                 360                 365
Ile Asp Gly Tyr Tyr Arg Leu Thr Ala Asp Ala His His Tyr Leu Cys
    370                 375                 380
Lys Glu Val Ala Pro Pro Ala Val Leu Glu Asn Ile His Ser Asn Cys
385                 390                 395                 400
His Gly Pro Ile Ser Met Asp Phe Ala Ile Ser Lys Leu Lys Lys Ala
                405                 410                 415
Gly Asn Gln Thr Gly Leu Tyr Val Leu Arg Cys Ser Pro Lys Asp Phe
            420                 425                 430
Asn Lys Tyr Phe Leu Thr Phe Ala Val Glu Arg Glu Asn Val Ile Glu
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         435                 440                 445
Tyr Lys His Cys Leu Ile Thr Lys Asn Glu Asn Gly Glu Tyr Asn Leu
    450                 455                 460
Ser Gly Thr Asn Arg Asn Phe Ser Asn Leu Lys Asp Leu Leu Asn Cys
465                 470                 475                 480
Tyr Gln Met Glu Thr Val Arg Ser Asp Ser Ile Ile Phe Gln Phe Thr
                485                 490                 495
Lys Cys Cys Pro Pro Lys Pro Lys Asp Lys Ser Asn Leu Leu Val Phe
            500                 505                 510
Arg Thr Asn Gly Ile Ser Asp Val Gln Ile Ser Pro Thr Leu Gln Arg
        515                 520                 525
His Asn Asn Val Asn Gln Met Val Phe His Lys Ile Arg Asn Glu Asp
    530                 535                 540
Leu Ile Phe Asn Glu Ser Leu Gly Gln Gly Thr Phe Thr Lys Ile Phe
545                 550                 555                 560
Lys Gly Val Arg Arg Glu Val Gly Asp Tyr Gly Gln Leu His Lys Thr
                565                 570                 575
Glu Val Leu Leu Lys Val Leu Asp Lys Ala His Arg Asn Tyr Ser Glu
            580                 585                 590
Ser Phe Phe Glu Ala Ala Ser Met Met Ser Gln Leu Ser His Lys His
        595                 600                 605
Leu Val Leu Asn Tyr Gly Val Cys Val Cys Gly Glu Glu Asn Ile Leu
    610                 615                 620
Val Gln Glu Phe Val Lys Phe Gly Ser Leu Asp Thr Tyr Leu Lys Lys
625                 630                 635                 640
Asn Lys Asn Ser Ile Asn Ile Leu Trp Lys Leu Gly Val Ala Lys Gln
                645                 650                 655
Leu Ala Trp Ala Met His Phe Leu Glu Glu Lys Ser Leu Ile His Gly
            660                 665                 670
Asn Val Cys Ala Lys Asn Ile Leu Leu Ile Arg Glu Glu Asp Arg Arg
        675                 680                 685
Thr Gly Asn Pro Pro Phe Ile Lys Leu Ser Asp Pro Gly Ile Ser Ile
    690                 695                 700
Thr Val Leu Pro Lys Asp Ile Leu Gln Glu Arg Ile Pro Trp Val Pro
705                 710                 715                 720
Pro Glu Cys Ile Glu Asn Pro Lys Asn Leu Asn Leu Ala Thr Asp Lys
                725                 730                 735
Trp Ser Phe Gly Thr Thr Leu Trp Glu Ile Cys Ser Gly Gly Asp Lys
            740                 745                 750
Pro Leu Ser Ala Leu Asp Ser Gln Arg Lys Leu Gln Phe Tyr Glu Asp
        755                 760                 765
Lys His Gln Leu Pro Ala Pro Lys Trp Thr Glu Leu Ala Asn Leu Ile
    770                 775                 780
Asn Asn Cys Met Asp Tyr Glu Pro Asp Phe Arg Pro Ala Phe Arg Ala
785                 790                 795                 800
Val Ile Arg Asp Leu Asn Ser Leu Phe Thr Pro Asp Tyr Glu Leu Leu
                805                 810                 815
Thr Glu Asn Asp Met Leu Pro Asn Met Arg Ile Gly Ala Leu Gly Phe
            820                 825                 830
Ser Gly Ala Phe Glu Asp Arg Asp Pro Thr Gln Phe Glu Glu Arg His
        835                 840                 845
Leu Lys Phe Leu Gln Gln Leu Gly Lys Gly Asn Phe Gly Ser Val Glu
    850                 855                 860
Met Cys Arg Tyr Asp Pro Leu Gln Asp Asn Thr Gly Glu Val Val Ala
865                 870                 875                 880
Val Lys Lys Leu Gln His Ser Thr Glu Glu His Leu Arg Asp Phe Glu
                885                 890                 895
Arg Glu Ile Glu Ile Leu Lys Ser Leu Gln His Asp Asn Ile Val Lys
            900                 905                 910
Tyr Lys Gly Val Cys Tyr Ser Ala Gly Arg Arg Asn Leu Arg Leu Ile
        915                 920                 925
Met Glu Tyr Leu Pro Tyr Gly Ser Leu Arg Asp Tyr Leu Gln Lys His
    930                 935                 940
Lys Glu Arg Ile Asp His Lys Lys Leu Leu Gln Tyr Thr Ser Gln Ile
945                 950                 955                 960
Cys Lys Gly Met Glu Tyr Leu Gly Thr Lys Arg Tyr Ile His Arg Asp
                965                 970                 975
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Leu Ala Thr Arg Asn Ile Leu Val Glu Asn Glu Asn Arg Val Lys Ile
            980                 985                 990
Gly Asp Phe Gly Leu Thr Lys Val Leu Pro Gln Asp Lys Glu Tyr Tyr
        995                1000                1005
Lys Val Lys Glu Pro Gly Glu Ser Pro Ile Phe Trp Tyr Ala Pro Gln
   1010                1015                1020
Ser Leu Thr Glu Ser Lys Phe Ser Val Ala Ser Asp Val Trp Ser Phe
025                1030                1035                1040
Gly Val Val Leu Tyr Glu Leu Phe Thr Tyr Ile Glu Lys Ser Lys Ser
               1045                1050                1055
Pro Pro Val Glu Phe Met Arg Met Ile Gly Asn Asp Lys Gln Gly Gln
           1060                1065                1070
Met Ile Val Phe His Leu Ile Glu Leu Leu Lys Ser Asn Gly Arg Leu
       1075                1080                1085
Pro Arg Pro Glu Gly Cys Pro Asp Glu Ile Tyr Val Ile Met Thr Glu
   1090                1095                1100
Cys Trp Asn Asn Asn Val Ser Gln Arg Pro Ser Phe Arg Asp Leu Ser
105                1110                1115                1120
Phe Gly Trp Ile Lys Cys Gly Thr Val
               1125
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGCGGAAGT GCTCTCGGCG GAAG 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTGTGCTAC AGTGCTGGTC GTCG 24